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[GANN, Vol. 40; March, 1949]

THE YOSHIDA SARCOMA, AN ASCITES TUMOR

(With Plates I and II)

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I. INTRODUCTION

(Considerations on living condition of malignant cells)

In advance it may be briefly noted how I have been led to deal with such a singular growth as an *ascites sarcoma* or, as I should like to name generally, a *fluid tumor*.

From the morphological standpoint the malignant growth is usually studied on its tissue architecture, but it must be analyzed into individual cells, in order to know under what conditions its integral elements are living or can live. Starting from investigations on the *stroma* of malignant tumors I have come to interest myself in an assumption that malignant cells, notwithstanding their origin, are capable of living and proliferating just like unicellular organisms without forming any tissue structure.

In our studies on transplanted tumors it has been demonstrated that in earlier stage of their development the tumor cells are proliferating in a state of suspension in tissue fluid or locally over-flowed blood[1]. But this condition is not limited to transplanted tumors. For example, within the *nests of carcinoma* the cells are suspended in the tissue fluid, for inside the nests there is no fibrous matter supped the cells. Also in metastasis of tumors it is an usual finding that in the dilated sinus of lymph nodes the tumor cells are proliferating completely in the form of cell suspenions. Carcinomas in man, especially those of younger persons, make in time surprisingly wide-extending metastasis and progress very acutely. In such cases the tumor cells, instead of forming solid nodules, fill up the lymph vessels and infiltrate diffusely. Jarchow[2] has commented on 49 cases of such carcinoma in the literature and proposed a general term *diffusely infiltrative carcinoma*. In all these facts it seems to be indicated that for cells which have once changed malignant, the formation of circumscribed nodules or histologically prescribed tissue architecture is in no wise a a matter of necessity.

Although the term *tissue* is applied in variable extent of meaning, accepted in its extreme, it consists of cells and intercellular substance which is proper to

them. In bone, cartilage, or connective tissues the cells exist embeded in hard or elastic intercellular substances respectively, and they present concrete or limited tissue formation. On the contrary, as to blood, lymph, or peritoneal fluid the intercellular substance is fluid, in which the cells are suspended. They may be called *fluid tissues*.

Accepting the word tissue as above mentioned, a malignant tumor is, or at least can be, in the nature of fluid tissue. If it does not commonly appear as such, it is because of the *stroma* which is by no means to be regarded as an undeprivable constituent of a malignant growth. It may be therefore possible, by removing the undesirable stroma, to transform a malignant tumor into a fluid tissue, in which only *parenchyme cells* proliferate in the form of suspension. If an animal tumor could be thus transformed and a new strain of transmissible *fluid tumor* presented, it may serve much to researches on cancer. A precedent is the "Ascites-Krebes" of Hesse (1927) and Loewenthal (1930)[3].

I have made under collaboration of *Z.* Sasaki many attempts to realize an ascites carcinoma in mice or rats by repeating transmission of tumor emulsion into abdominal cavity, but in vain. We have learned that one must restrain from too wide extension and indiscriminate application of the assumption of fluid tumor. But one must be convinced of the fact that malignant cells are deviated from normal living condition of tissue cells, namely that they are deprived of their property relating to the intercellular substance. In fact they can principally live on and proliferate at any site in the body where they have been deposited in metastasis formation.

The *epithelial* cells of a normal tissue are interposed between outside world (external environment) and internal fluid environment of the body. In each of the cells the distal end is in contact with outside world, directly (e.g. in digestive or respiratory tracts) or indirectly (e.g. in acini of gland), while the proximal end is bathed in the tissue or intercellular fluid[1]. However, in the condition of malignant growth they are deprived of the contanct or communication with outside world. In sections of cancer originated from glandular tissue there are seen many gland-like epithelial formation with lumen. But they have in no wise communication with the duct. They are isolated from normal gland tissue and embedded in the deep layer of body. They are only a tissue-imitating formation of infiltrative growing cell groups. In further stage the cancer cells are separated one by one, each being all over the cell surface in contact with the tissue fluid, i.e. suspended in the fluid. In fact the so-called *emancipation* of cells is a criterion of recognizing the very beginning of malignant change in any neoplastic growth.

A consideration must be paid concerning the epithelia of endocrine glands, which already in normal condition do not in any way communicate with the

outside world. The thyroid gland offers in this connection an interesting example. In a condition of pathological cell proliferation of this gland, the epithelia infiltrate or invade very soon after the onset of the condition into surrounding tissues or even into blood vessels, notwithstanding still benign general histological appearance. It is known in man as *metastasizing adenoma* of thyroid. My former experiments with o-Amidoazotoluol have demonstrated that administration of this substance brings about a very active proliferation of follicular epithelia of thyroid gland. In one case of rat fed on this substance I observed an active invasion of the proliferating cells into adjacent veins only 36 days after the beginning of the treatment. Such a phenomenon may serve as a start of consideration that the epithelia of the gland are, at least in one point, already in physiological condition common with malignant cells.

Concerning the sarcoma cells originating from non-epithelial cells, it is noticeable that they live on for a long time in a condition of immediate contact with blood. As already demonstrated by us[5], there are in sarcoma tissue plentiful blood vessel-like formation within which the blood actually streams. But they are not true blood vessels equipped with histologically prescribed wall constitution. Their wall is constructed from very sarcoma cells. This is also a morphological expression of the fact that the cells, when once changed malignant, are essentially changed in their behaviour to living environment.

All things mentioned may support principally the assumption that the malignant growths, provided that they are truely malignant, not only in clinical sequences but also in strict acceptance, are principally in the nature of fluid tissue.

The present tumor, an ascites sarcoma, originated in an albino rat, presents a specimen of the fluid tumor. It was discovered by us in 1943 in Nagasaki and has ever since been continuously transplanted, being employed in various ovservations and experiments.

II. On the Yoshida Sarcoma

(1) Origin of the tumor

In Pathological Institute of the Nagasaki Medical College M. Muta[6] has carried out at my suggestion a series of experiments on effects of successive superposition of various carcinogens. In one of his experiments a group of 20 albino rats was at first fed on o-Amidoazotoluol for 3 months just after the method of hepatoma-producing experiment described by Sasaki and Yoshida[7], then this was stopped and followed immediately by cutaneous application of potassium arsenite solution prepared by addition of equal amount of absolute alcohol to Fowler's solution. The mixture was applied with a painting brush three time a week locally to hair-clipped skin on the backs of the animals.

In the course of 4—5 months of the arsenite application, there developed in several animals malignant hepatoma in the liver. The result approximates that of continuous administration of sole o-Amidoazotoluol throughout 7—8 months.

It is well known that even if o-Amidoazotoluol acts carcinogenic upon the liver, only 3 months are not sufficient to prove its definite action. It needs at least about 7 months to realize a malignant hepatoma. In 3 months it brings about in the liver only diminutive changes, from which, when left without further treatment a hepatoma does not generally develop. And, as for the arsenite, it is a substance of minimal carcinogenicity, if at all, and especially it does not act carcinogenic upon the liver cell. Then, it may be safe to conclude from the result of this experiment that two different *carcinogens*, each of which is either unsufficient or unable severally to incite a carcinoma, are able, when successively superposed, to present a collective effect as to accomplish a development of real carcinoma.*

Now, in one of the twenty rats, at the end of the 3rd month of arsenite treatment, an unexpected neoplastic growth was detected within the scrotum. It seemed that the right side testicle was swollen as a tumor infiltrating into surroundings and adhering with scrotal skin. After about a month the tumor threatened to ulcerate. The animal was killed for autopsy. A malignant growth was disclosed showing widely spreading continuous infiltration from the surroundings of both testicles to retroperitoneal tissue, as to be seen in Fig 1. Testicles were left uninvaded, but showed pressure atrophy.

In the peritoneal cavity, there was found a milky ascites which appeared somewhat different from purulent matters. It was really a suspension of tumor cells. The milky fluid was transplanted intraperitoneally into two rats, of which one took. Successive intraperitoneal transmissions of the tumor were continued up to now, amounting to over 200 generations.

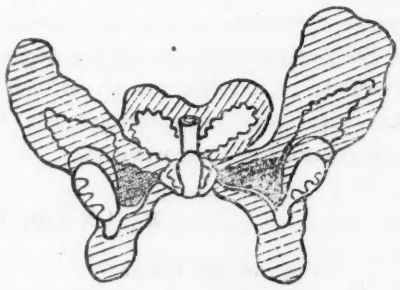

Fig. 1. Diagram of cell infiltration in the primary tumor.

On the other hand, pieces of tumor tissue which presented microscopically a typical feature of round cell sarcoma (Figs. 1—2 in Plate I), were grafted subcutaneously in three rats, and in one of them developed at the site a large massive tumor just like the usual sarcoma. But, when a piece of the subcutaneous

* The same result was obtained by application of coal tar instead of arsenite. Also experiments in mice resulted all the same. Detailed paper of this experiment will appear later in this journal.

tumor was introduced into there peritoneal cavity, there arose again milky fluid growth. And inversely, a droplet of ascites, when brought into subcutaneous tissue, presented always a massive solid tumor. The features of this tumor, either solid or fluid, depend merely upon the preexistent conditions of the site where the cells are introduced, and neither of them can be regarded as the original figure of this growth, each being only a transcient appearance. We have therefore kept up the strain in the fluid form, from a droplet of which a solid tumor can be obtained at any time when wanted.

Provisionally I called this new strain of sarcoma *ascites sarcoma* or *ascites sarcoma of Nagasaki strain*[5]. In 1948, at general meeting of Japanese Cancer Association it was proposed by R. Kinoshita, President of the society at that time, to call the present tumor *Yoshida sarcoma* and was accepted.

A question about the originating site of the tumor is left open. In fact, we have at first marked it within the scrotum, but an assumption that it might have originated within the abdominal cavity, is not to be excluded, for the tumor cells, when introduced into the cavity, infiltrate and spread always in the same way, presenting a feature which is closely similar to that of the original tumor. Also the cause of this tumor, whether purely spontaneous or the administration of o-Amidoazotoluol or arsenite has had any influence upon its development, is left undecided. But it must be pointed out that such a peculiar tumor as the present one has never been reported from any laboratory, in spite of the fact that the albino rat is an animal which is employed everywhere in large number.

(2) Tumor cells

1 cmm of the ascites contains apprxomately 1 milion tumor cells, which show in Giemsa-stained smear characteristics as follows:

i) They are larger than any normal cells which appear in the blood or peritoneal cavity. ii) The nuclei are oval or kidney-shaped and are biassed on one side of cytoplasm, equipped with large and clear uncleoli. In the wider side of cytoplasm there are abundant azurophile granules in rosette form, so-called azurosette (Fig. 2 in text and Fig. 9 in Plate II).

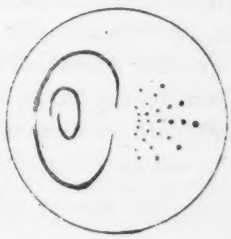

Fig. 2.
A diagramatic representation of the tumor cell.

By these criteria the tumor cells are distinguished with ease. (Figs. 4—10 in Plates I—II).

(3) Technique of transplantion

The technique is of the simplest. We prepare a sharp pointed capillary pipette

shaped as seen in Fig. 3. The point, sterilized in flame, runs into the abdominal cabity, from where the ascites is naturally drown up into the capiallary in amount of about 0.05 cc. The fluid obtained is blown into the peritoneal cavity of a new host from the same capillary. A capillary content can be divided for 2 or 3 animals. Hence the amount of cells transmitted varies by cases from about 10 to 50 milions. During this procedure smears for microscopical examination are prepared from a droplet of the ascites. Larger doses of ascites, say 0.5 or 1.0 cc, do no harm to transplantation but it is not necessary.

Fig. 3

This simple method, a device of H. Sato, is of course applied also for the purpose of examination of the ascites at any time. It can be repeated as often as wanted without distressing the animals. It is also readily available for the examination of normal peritoneal fluid of rats or mice, with the same easiness and effectiveness.

The ascites, kept in ice chamber, keeps its transplantability for 9 days at least. But at -200°C in liquid air it is lost in less than 10 minutes.

(4) Rate of transplantation

Throughout over 209 generations in five years the average of takes was 98 %. In 55 cases in which it was microscopically confirmed on the next day of teh transplantation by examinations of the peritoneal fluid that the tumor cells had been really introduced, 54 proved positive, hence also 98 %. This percentage remains constant from the very beginning of the successive transplantation until now. As for the remaining animals of 2%, it has been demonstrated by repeated transplantations in the same animals at various time intervals that they are invariably insusceptible to the tumor. It must be noted that the rats used do not belong to any genetically pure strain.

In older animals of over 200 g body weight the rate appears somewhat to decrease, but this is not sure. We employ usually 70—120 g rats, recognizing no difference between male and female. In cases of subcutaneous transplantation of the ascites, if suppuration occurs at the site, the rate is reduced in accordance.

(5) Life days of tumor animals

The term from transplantation to the death of animals is very short, a natural regression of the transplanted growth occurring never. The average is a little more than 12 days, the extreme being 24 days in maximum and 8 days in minimum. The majority of animals die about in average term, of wide-extending malignant invasion and increasing ascites production which sometimes amounts to 10 cc.

(6) **Tumor cells in streaming blood.**

As this tumor closely resembles leukemia in its behavior and general impression, findings in blood, bone marrow and associated organs have been kept with care, but without presenting any remarkable changes. In blood and bone marrow some tumor cells are found in end stage of animals, but very few in number. In 5 cases number of tumor cells in streaming blood had been counted, amounting to 0.4, 0.8 (2 cases), 1.0, and 2.5 % of the total number of blood leucocytes.

(7) **The minimal number of cells necessary for transplantation.**

In spite of the inferiority in percentage of tumor cells in blood, attempts to transmit the tumor by intraperitoneal introduction of the blood resulted positive. The number of the cells transmitted in these cases was estimated to be about 30,000 in maximum and 8,000 in minimum. As it has been hereby demonstrated that so great a number of cells as over 10 millions is not necessary and about 8,000 have proved to be enough for effective transplantation, attempts were made to know the minimal number of cells necessary for the transplantation:*

Very small droplets of supernatant of diluted tumor ascites were examined under the microscope and actual number of cells contained in them counted. Then, by the aid of micromaniplator several of the droplets were sucked up into a micropipette until the amount of the fluid became sufficient for the transplantation, controlling at the same time the number of cells within the capillary. A transplantation by this capillary nethod results, as is evident, never in a transmission of no more cells than really calculated.

Transplantation with more than 20 cells succeeded almost constantly; with 11 also succeeded; with 4 and 5 cells failed; but, with 2 cells and with a single cell it succeeded each in one case out of two and three trials respectively.

In cases of transplantation with a few cells, especially with a single cell, it is of course a matter of chance, to catch a cell of full potency in further proliferation, as a single cell suspended in the fluid can be a degenerated cell. Moreover, it can be a normal cell such as leucocyte, lymphocyte or monocyte.

Hand in hand with these experiments it was tried to transmit the tumor with fluid completely free from visible cells, but the result was decided to be negative.

In this connection it must be noted that investigations by Sato and Nagasawa, who attempted to transmit the tumor with filtrates of the ascites by Berkefeld's or Seitz's filter, have demonstrated that the cellfree filtrate is completely incapable of transmitting the tumor.

In the cases of transplantation with a small number of cells the days needed

* Details will be reported by Ishibashi in this journal.

for growth of the tumor, i. e. the days until the peritoneal cavity presented a state of pure culture of tumor cells were 15–20 days, evidently prolonged as compared with 3–4 days in the usual transplantation in which 10–50 millions of cells are transfered. But, a prolongation more than about 3 weeks is not the case. Even in the case of a single cell it was 18 days.

(8) Infiltration of tumor cells into the tissue.

As already mentioned, the characteristic of this sarcoma is that the intraperitoneally introduced tumor cells proliferate as an ascites tumor, but on the other hand, especially at the later half of the their growth they infiltrate lively into the tissue of the host, revealing their nature of real malignancy. (Fig. 1-3 in Plate I).

Features of the infiltration are:

i) When they infiltrate into the liver or spleen they enter from the hilus of the organs and make their way along adventitial tissue of blood vessels or ducts. They do not invade the peritoneal surface of an organ. Only in kidney an infiltration from the cortex can occur, as the cells enter from areas where the organ surface is saved from peritoneal covering.

ii) The infiltration spreads over very wide extent, but it presents always a form of continuous flat infiltration without forming any localized nodular mass. Something like a spreading factor appears to act in the propagation of this tumor.

iii) Distant metastasis by lymph or blood stream are seldom to be found, perhaps depending on the brief life span of the tumor animals.

(9 Division of tumor cells.

Mitosis of the tumor cells within the ascites is very abundant. They are observed in the Giemsa-stained smear of the ascites in fine figures and in every phase of mitosis. (Fig. 5 in Plate 1 and Fig. 9 in Plate II). As for the number of chromosomes of tumor cells, after investigation of Sato and Shinagawa, 32-36 make the majority, while 42 is the normal in rat.

Bi-nucleate cells are plentiful in this tumor, and tri- or more nucleate ones are not rare. It is evident that these polynucleate cells owe their appearance to segmentation of a single nucleus, but it is very doubtful if a direct division of a nucleus could result an actual multiplication of cell, because we have never met with a division of cytoplasm which accompanies a simple cleavage of nucleus. In this tumor, at least, it is rather safe to say that actual multiplication of cells depends exclusively upon the mitotic division, while amitotic division produces only polynucleate cells.

Multipolar divisions are also often. Tri- or tetrapolar divisions are often met

with. But it is noticeable that both two nuclei of a binucleate cells enter very often simultaneously into prophase so as to result a situation which is in its appearance indistinguishable from that of tetrapolar division.

It has been demonstrated by investigation of Atsumi that the mitosis predominates in the earlier half of the tumor growth, while in the later half the amitosis surprisingly increase in number.

(10) Neutral red rosette and others.

Supravital staining with neutral red presents an excellent figures of neutral red rosette which very closely resemble that of monocyte. This is one of the two findings which lead us to an assumption that the mother cell of this tumor may be monocyte (see below). Janus green granules which surround the neutral red rosette and are distributed difusely over the cytoplasm are not always distinct.

Phagocytic activity of tumor cells, tested by intraperitoneal introduction of carmine solution or China ink, is neglectably small, although conditions have occasionally been observed in which real tumor cells in the ascites are phagocyting degenerated leucocytes or probably degenerated tumor cells.

Oxidase and peroxydase reactions have proved negative in the tumor cells.

(11) Morphology of the tumor cells dynamically described.

The morphological properties of the cells of this tumor have been schematically described above (Fig. 2), but the appearance of a cell is not changeless throughout its life which begins at the reconstruction of nucleus in the telophase of mitosis. A cell ripens or grows, degenerates or superannuates, when it does not devide at any due term. Its appearance changes in accordance with this. The above

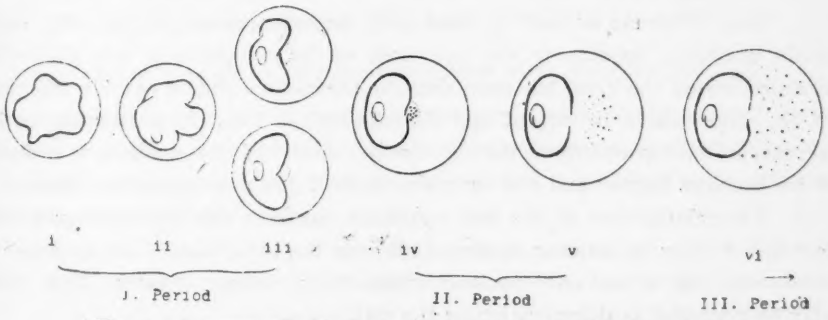

Fig. 4. A tumor cell ehanges its appearance in the direction: i→vi

described is therefore a schema of a tumor cell composed of some distinguishable marks which was picked up from various cells. It is useful and convenient to

inform about yet unknown tumor cells, but in order to make known the real aspect, a dynamical description of the cell in its changing figures throughout its life is needed. The analogy is to be seen in the histology of endometrium which is in continuous movement of periodical changes.

Although in usual tumors such a description is almost impossible, the present tumor, as easily to be seen, favours an attempt in this respect. Results I have obtained in my observation may be summerized as follows (Fig. 4):

i) In the stage of nuclear reconstruction the nucleus is situated in the middle of the cytoplasm.

ii) Then the membrane of nucleus which is filled up with diffusely distributed fine granules of chromatin, becomes clear. The nucleus is thereby completed and shows at this time an evidently lobulate formation which resembles closely that of monocyte. This leads us, together with the above mentioned neutral red rosette, to an assumption that the mother cell of the present tumor may be monocyte. The nucleus remains yet in the middle of the cytoplasm and the nucleolus is not yet distinct.

iii) The lobulate structure of the nucleus is lost gradually, approaching a globular or ovoid form with smooth surface. Meanwhile the nucleus changes its location from the midde towards one side of the cytoplasm; The nucleus becomes kidney shaped, equipped with a depression towards the wider side of cytoplasm, where closely touching the nuclear depression, a pale stained small area is seen.

iv) The cytoplasm enlarges further, the nucleus becoming thicker. The appearance of the cell resembles the "histiocyte", especially the cell which is usually called histocyte in the peritoneal fluid, distinguishing itself from the latter in the deep stained cloudy cytoplasm. Moreover, a tumor cell is always larger than any normal cells.

v) Then, following or hand in hand with the enlargement of the cell, azurophile granules, appears in the pale area of the cytoplasm at first distinctly localized within the area, but soon disperse themselves widely outside the area so the azur-rosette is formed and the pale area is lost. In proportion to the increase of azur-granulation, the chromatin content of the nucleus is reduced which becomes lighter and two or more nucleoli disclose themselves plainly.

vi) The enlargement of the cell continues further; the azurophile granules increase further in number dispersed all over the cytoplasm. The nucleus becomes still lighter and only nucleoli within them become clearer. This state may be regarded as degeneration of the cell.

The continuously changing phases of the tumor cells and the direction of the change are not only confirmed by observations of transitient cell forms in a smear, but also known by serial examination of the tumor ascites throughout

the whole course of the tumor growth from its very beginning until the death of the animal. In the early stage of the growth (3-4 days after the transplantation) the majority of cells belong to the type of i-iii (1st Period); in the middle stage (5-8 days) to iv-v (2nd Period, and in the end stage to vi including more worn out cells (3rd Period).

In the 1st Period there appears a little polymorphism of tumor cells, consisting of uniform cells not yet with azur-granulation (Fig. 1-2 in Plate I). But, as it proceeds through the 2nd period (Fig. 7 in Plate II) towards the 3rd Period polymorphism and atypia of cells becomes obvious.

It may be notable that the consequence of the transplantation, no matter whether the transplant may originate from 1st Period ascites or 3rd Period ascites, is always the same. In other words, the ascitestumor which grows in any host develops always in the same way, completely independent of stages of transplant-ascites from which it has originated, and the regular sequence of the above mentioned three periods of development can be proved in all instances. The polymorphism or atypia of cells in later stage is not, therefore, a phenomenon which can bedelivered as itself to the next tumor generation, being only an appearance in the end stage of tumor within an individual host, i. e. it means in no wise the anaplasia of tumor cells.

(12) Azur-rosette.

As this is one of the most distinguishing characters of the present tumor cells, attentions have been paid thereto. After all, we have interest in following two points: 1) The azurophile granules are apparently the product of nucleus, i. e. they seem to be ejected from within the nucleus into the cytoplasm. Hand in hand with the increase of granules the chromatin content of nucleus diminishes (Fig. 8 in Plates II). 2) The granules can again disappear in an individual cell, after they have been produced.

(13) Cellular reaction of the peritoneal fluid upon the transplantion.

Reaction of the peritoneal fluid upon the tumor transplantation may deserve special mention, as in the fluid, compared with the tissue reaction in ordinary cases of subcutaneous transpantation, the reaction is analyzed more easily and in detail into its cellular composition.

To mention at first conclusively, the leading elements of the reaction are neutrophiles and monocytes. Though the emigration of the leucocyte is very lively, it is a prompt reaction which reaches to its extreme only in about two hours after the transplantation, and decrease continually in 10-20 hours. Following the cease of the neutrophile reaction the monocytes increase in number displaying phagocytic activity, which continues for about 24 hours.

After the investigation of Sato and Ishibashi in my laboratory the normal constituents of the peritoneal fluid of rat are following four elements: 1) the monocytes (64-70 %), 2) the lymphocytes (14-17 %), 3) the eosinophile leucocytes (13 %), and 4) the mast cells (3-5 %). The neutrophile leucocyte is not an integral member. If the latter may be found in the fluid it should be considered that in sucking up the fluid some blood might have admixed thereinto, provided that no inflammatory process is going on within the peritoneal cavity.

In many instances the peritoneal fluid has been serialy examined every hour after the transplantation, and the progress of the reaction may be summerized as follows:

i) The neutrophiles which are normally absent in the peritoneal fluid appear already in 30 minutes and increase very rapidly, and in two hours they make the majority of the cells in the field. But they cease readily, showing pyknosis and karyorrhexis. A little delayed, i. e. at about the 3rd hour after the transplantation, the eosinophiles begin slowly to increase and continue to increase for twelve hours, until they show also pyknosis or karyorrhexis and other degenerative signs. But in the latter, compared with the former, neither the increse is so striking nor the progress of increase and decrease so abrupt. After all, for several hours in the beginning there are definite superiority of the neutrophiles in the field (Fig. 4 in Plate I), while about 10 hours after the eosinophiles become predominant.

ii) Following the decline of activity of the both leucocytes, i. e. about 12 hours after the transplantation the increase of monocytes, which has been slowly in motion, becomes striking and continues for further 12 hours. Hand in hand with the increase in number their bodies enlarge obviously presenting the appearance of macrophages, and in fact they phagocyte most actively the degenerated leucocytes of both sorts. But picture in which the tumor cells are phagocyted in them are very hard to find. The activity of macrophages (derived from monocytes) may be therefore regarded as a phenomenon in response to the degeneration of the leucocytes. In fact, as the leucocytes, especially the neutrophiles vanishes, the macrophages disappear as if to follow the trace of the former, and the monocytes diminish also.

iii) The proliferation of the tumor cells becomes abruptly obvious just at the time when the activity of the macrophages is at the height, and thereafter continues its way.

iv) Meanwhile, the lymphocytes remain settled. The mast cells disappear completely in 24 hours after the transplantation, and never come back.

After all, 48-72 hours after the transplantation, when the abdominal cavity has come to a state of pure culture of tumor cells, the cells which are scattered among them are monocytes, lymphocytes, and eosinophiles, though they are

very scanty compared with the tumor cells. (Fig. 5-6 in Plate I).

It is noteworthy that the reaction in the peritoneal cavity upon the transplantation proceeds so regularly as mentioned, successively alternating the cellular composition. And the tumor development which directly follows this reaction proceeds also very regularly showing the above described three periods. Owing to this regularity one can estimate almost correctly the hours or days after the transplantation of any unknown case by examination of smears of ascites. This may be favourable for employing this tumor in any experiment, because one may thereby notice very readily anything deviated or unexpected, either in the cellular composition or in the course of development.

(14) On the mother cells of tumor.

At present it seems to be natural to regard the monocyte as the mother cells of the present tumor. Those which chiefly support this assumption are the neutral red rosette in the tumor cells and lobulate form of nucleus seen in the early stage of cell development.

III. Actions of Various Chemicals upon the Yoshida Sarcoma (A fundametal research on chemotherapy of cancer)

The chemotherapy of malignant diseases has long been discussed and much work has been done. But, researches in this field are not yet systematized. Though many causes could be pointed out thereto, one of the greatest may be, as I think, the fact that an available indicator which serves to demonstrate exactly the effect of any substance tested has been incomplete or rather has been missing.

The routine method which compared in transplanted tumors the gross size of the tumors or speed in their growth after administration of any substance to be tested is not always adequate or reliable, because the judgement is unavoidably complicated by interposed conditions. As for tests of inhibiting actions of some substances upon cancer producing activity of the cancerogens the matter is also the same.

In investigation of therapeutic effects of chemicals, those which are desirable to know first of all are: i) their direct actions upon the malignant cells, and ii) the percentage of tumor cells affected by them. For this purpose it is necessary to have a test object in which it is possible to examine the tumor cells individually at any time both before and after the administration of substances to be tested. We have just lacked such a test object.

The Yoshida sarcoma appears to be capable of filling this lack, for it is possible in this sarcoma to examine the tumor cells by the smear of the ascites at any

time desired, and at any number of intervals after administration of substances by whatever the way, e. g. subcutaneous, intravenous, or intraperitoneal. Moreover the toxicity of the substances upon the animal body is at the same time taken into consideration.

From this point of view we have carried out a series of experiments in which we have employed more than 45 substances as shown in Table 1. Some of them are known to have some inhibiting effects on cancer.

Table 1. Chemicals tested.

Colchicine, Sinomenine, Apomorphine, Thebaine, Fritillarine, Verticine, Verticilline, Veratrine, Papaverine, Dihydropapaverine, Hydrastine, Hydrocotarnine, Acenaphthene, Cacodylic acid, Potassium arsenite, Atoxyl (and 6 other Phenylarsine derivatives), Neo-Salvarsan (Neo-Natvarsan, Neo Arsenobenzol), Neo-Neo- Salvarsan (Neo-Neo- Natvarsan, Neo-Neo-Arsemine), Mapharsen, Trypaflavine, Rivanol (Rimaon, Hectaline)

Chloralhydrate, Urethan, α-Dinitrophenol,

Furfural, Anthranilic acid, Cephalantine, Benzochinon, o-Amidoazotoluol, Butter yellow, Pellidol, Phenanthrene, Carbazol, H_2O_2, $KMnO_4$.

(Note: Underlined substances have shown definite effects.)

Method of experiment: Principally disolved in water, each substance was at first injected directly into the peritoneal cavity, and the effects were then compared with those by subcutaneous injection. But the former formed the majority, especially in cases of the substances of high toxicity. Difficultly soluble substances were introduced in the form of suspension directly into the peritoneal cavity. The treatment was undertaken in every case at the height of tumor growth, i. e. about 5-6 days after the transplantation. At 1, 3, 6, 9, 12, and 24 hours after the injection smears of the ascites were examined stained by Giemsa. After 24 hours the ascites were examined once a day.

Results: Out of 45 substances tested 7 have proved to have definite effects upon the tumor, which consist chiefly in destruction of tumor cells and prolongation of the life of animals. (Table 1). Results may be summarized as follows:

1) Although the majority of the substances do not show any definite action, some of them produced violent destruction of the tumor cells. And in the manner of the destruction there are considerable differences among various substances. (Fig. 10-12 in Plate II).

2) With some effective substances the ascites becomes in 6-12 hours almost clear, in which almost nothing but cell fragments is to be seen. But by careful examination it will be demonstrated that a few, at times very few, tumor cells which have preserved their constitution almost intact survive scattered in detritus. This survival of a few cells is common for all substances, even for a very violent substance. (Fig. 12 in Plate II).

3) Though the survived cells remain for a time in a condition of pyknosis,

they recover in about 12 hours the lively condition and begin to divide, so there occurs a recidivation which proceeds just like a new transplantation.

4) Even in cases in which it was tried to repeat the injection in various time intervals it failed always to prohibit the survival of a few cells. This means that with a single substance all attempts to destroy the total cells have failed in the present experiment.

5) Though the effects on the tumor cells vary according to substances, effective substances injure commonly the nucleus, which breaks up and disappears after having shown various stages of degeneration. In some cases the nuclei lose first of all their colorability. (Fig. 11 and 12 in Plate II).

6) In doses by which the tumor cells are injured so severe as mentioned, especially in cases of intraperitoneal administration, an evident disturbance in the animal body is not perceptible. Moreover, as the number of tumor cells, even if temporalily, is reduced to an extreme, the animals get a condition of temporaly recovery, and this causes a prolongation of the life of animals up to twice or more than 12 days, the average days of life of untreated tumor animals.

Now, from results obtained in this preliminary experiment, it may be safe to conclude that the Yoshida sarcoma offers a favourable test object for the fundamental investigation of chemotherapy of cancer.

In this connection it may be worthy to note a research performed recently in my laboratory on this tumor. Nagata has made a series of experiment with culture filtrates (Shwarzman's filtrates) of various bacilli, of which those of both typhoid and dysentery bacilli have proved a remarkable healing effect upon the tumor*. The filtrates were injected directly into the peritoneal cavity once a day in the dose of 0.25 cc every time. After treatment of 9-10 days more than half of the animals recovered completely from the disease. In the remainder the life was prolonged by twice or more. In this experiment we were interested in the fact that throughout the healing process of the tumor visible destruction of the tumor cells were very slight, while emigration of neutrophiles into the peritoneal cavity reached the highest degree, and the tumor cells reduced day by day continually in number, to disappear completely in about 10 day.

Though an evident effect as mentioned has not been demonstrated by subcutaneous administration or in cases in which the treatment was started at the end stage of the growth, i. e. in the stage when the infiltration of the tumor cells had already widely extended, the effect of the culture filtrates upon the tumor should not be disregarded.

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* The paper will shortly appear in this journal.

Résumé

(1) Though the histology of cancer which deals mainly with relations between parenchyme and stroma of tumors has informed very little about living conditions of individual malignant cells, it is conceivable that malignant cells, when they enter into a place where there is no supporting fibrous element, are able to proliferate in a condition of suspension in the tissue fluid without accompaniment of any so-called stromareaction, e. g. in lymph vessels, lymph sinus, or within the nests of carcinoma. The malignant cells are capable of living on without depending on the stroma, which is, strictly speaking, by no means proper to a malignant tumor, represtenting rather the preexistent tissue constituents proper to the site where the malignant cells have lodged by chance and commenced to proliferate. Remove the stroma from the tissue of a malignant growth, there remains nothing else but individual tumor cells and surrounding tissue fluid. A malignant growth is therefore in its formal nature a cell suspension, a fluid tissue, provided that it is truely malignant, not only in clinical sepuences, but also in biological behaviours of parenchyme cells.

In many respects of cancer researches, especially in cytological studies or chemotherapeutic investigations, it is surely favourable to employ a malignant growth in fluid state without stroma formation.

(2) The Yoshida sarcoma, discovered by T. Yoshida, Y. Muta, and Z. Sasaki, in 1943, is a malignant fluid growth. It originated in an albino rat which had been fed with o-Amidoazotoluol for 3 months and then appliedcutaneously with 5 % arsenite solution for about 3 months. The tumor cells proliferate chiefly in tbe abdominal cavity forming ascites, of which 1 cm contains about 1 milion of tumor cells. Successive transplantations have been carried on very easily by introducing a droplet of the ascites into the peritoneal cavity of rats, amounting to over 200 generations until now, and is going on further. The rate of positive transplantation is 98 %. The tumor animals die in 12 days in average, of increasing ascites and widely extending infiltration of tumor cells into abdominal tissue. The ascites, introduced into subcutaneous tissue, causes a large solid tumor at the site, which presents histologically the figure of round cell sarcoma. Inversely, by introducing a piece of such tumor into the peritoneal cavity there arises again the ascites.

We have succeeded in trasmitting this neoplasm with a single cell (microscopically controlled), but without any cell it has failed. Presence of a virus has been until now in no wise demonstrated. The behavior of the tumor cells is similar to that of the monocyte.

(3) The smear of the ascites stained with Giemsa solution permits the examination of the conditions of the tumor very easily at any time wanted. More-

over the condions of the tumor cells can be discussed one by one in the smear. Taking advantage of this, effects of various chemicals upon the tumor has been tested and it has been demonstrated that the present tumor offers an excellent test object valuable for fundamental investigations of chemotherapy of cancer.

Calchicine, arsenic compounds, and several other substances have shown evident destructive actions upon the tumor cells. Culture filtrates of various bacilli, especially those of typhoid and dysentery have proved definite effect: they were injected directly into the abdominal cavity once a day, and in about 10 days more than half of the animals treated recovered completely from the disease.

References

(1) Yoshida, T., u. Kin, C.: Über den histologischen Aufbau des Impfcarcinoms, Gann, **37**, 343, 1943 (Japanese).

(2) Jarchow, S.: Diffusely infiltrative carcinoma, a hitherto undescribed correlation of several varieties of tumor metastassis. Arch. Path. **22**, 674, 1936.

(3) Hesse, F.: Über experimenellen Bauchfellkrebs bei Ratten. Centralbl. f. Bakt. I. Abt. **102**, 367, 1927.
Lowenthal, H., u. Jahn, G.: Übertragungsversuche mit carcinomatöser Mäuse-Ascites flüssigkeit und ihre Verhalten gegen physikalische und chemische Einwirkungen. Z. Krebsforsch. **37**, 1932, 439.

(4) Cowdry, E. V.: Text Book of Histology, 1934, 19-20.

(5) Yoshida, T., Shimauchi, T., Tsurusaki, H., u. Sasaki, Z.: Über das Stroma des Sarcoms. Gann, **37**, 1943, 341 (Japanese).

(6) Muta, T.: Kombinationsversuch der carcinogenen Wirkungen verschiedener Substanzen. Gann, **37**, 343, 1943, 298. (Japanese)

(7) Sasaki, T., u. Yoshida, T.: Experimentelle Erzeugun des Lebercarcinoms durch Fütterung mit o-Amidoazotoluol, Virch. Arch. **295**, 1935, 175.

(8) Yoshida, T., Muta, Y., u. Sasaki, Z.: Studien über das "Ascites-Sarcoma" (I). Proc. Imp. Acad. Tokyo, **XX**, 1944, 611. ; A special report on "studies on the ascites sarcoma of Nagasaki strain" read by Yoshida at the General Meeting of the Japanese Patholcgical Society in 1947.

Legend for Plate I.

Fig. 1 and 2: Various features of the tumor in a section of the primary tumor. In Fig. 1 the cells are very large in size resembling the "rethotelsarcoma"; in Fig. 2 they are small resembling common round cells sarcoma. (Both figures enlarged equally).

Fig. 3: Infiltration of the tumor cells throughout the diaphragm. (16 days after the transplantation of the ascites).

Fig. 4: (Smear of ascites; Giemsa) 5 hours after the transmission. Mitosis of tumor cells introduced (M); young tumor cells with lobulated nuclei (J); large number of neutrophiles (n). t: tumor cells. e; eosinophiles.

Fig. 5: (Smear of ascites; Giemsa) 48 hours after the transplantation. Already a state of pure culture of cells. Large number of mitosis in various phases. Typical appearances of tumor cells. In some of them the azur-rosette. m: monocytes; all others are tumor cells.

Fig. 6: (Smear of ascites; Giemsa) 4 days after the transplantation. A group of cells in the 1st period. l: lymphocytes; all others are tumor cells.

Legend for Plate II.

Fig. 7: (Smear of ascites; Giemsa) General view of the ascites on 9 th day after the transplantation. Most cells are in the II nd period showing azur-rosette. T: two cells in telophase.

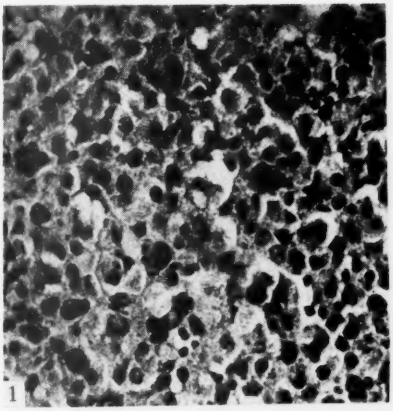
Fig. 8; (Smear of ascites; Giemsa) Azur-rosette in a tumor cell. l: lymphocyte, m: monocyte.

Fig. 9: (Smear of ascites; Giemsa) Mitosis of the tumor cells.

Fig. 10: (Smear of ascites; Giemsa) 6 hours after subcutaneous injection of 0.2 mg colchicine. Arrest of mitosis in metaphase and scattering of destructed chromosomes. Also injuries of other tumor cells with resting nuclei.

Fig. 11: (Smear of ascites; Giemsa) 12 hours after intraperitoneal injection of Neo-Neo-Arsemine (0.6 cc of 4.5: 100 solution). Destruction of tumor cells.

Fig. 12: (Smear of ascites; Giemsa) 1 hour after intraperitoneal introduction of Dihydropapaverine in suspension. Various stages of nuclear destruction. A cell remains yet intact.



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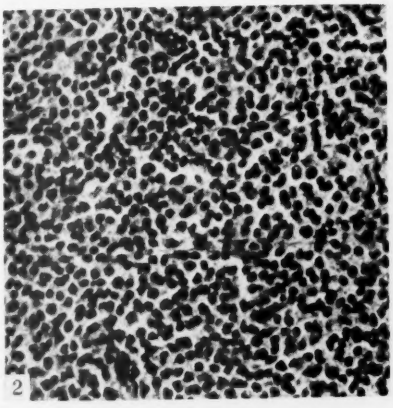

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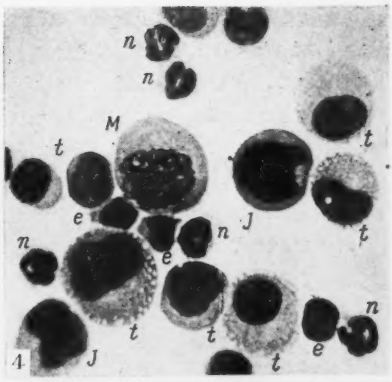

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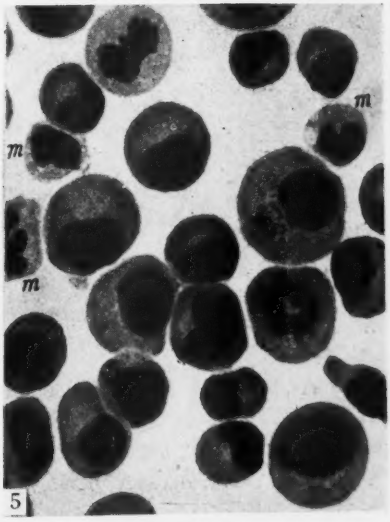

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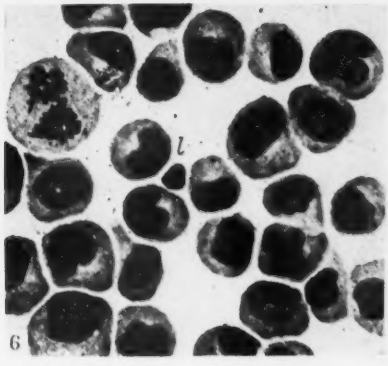

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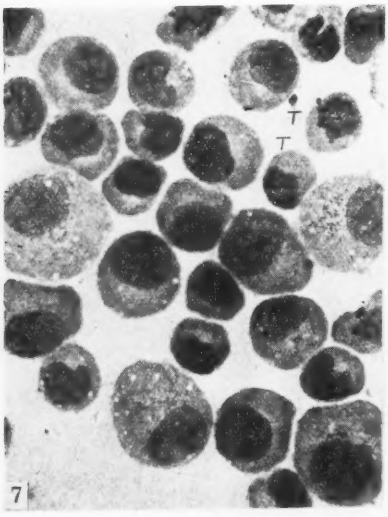
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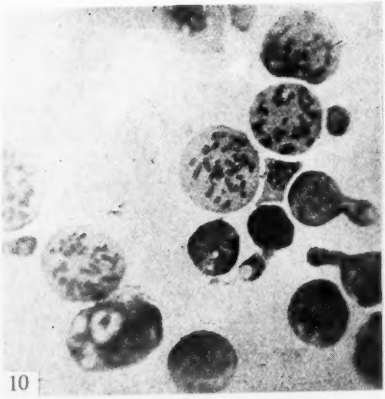
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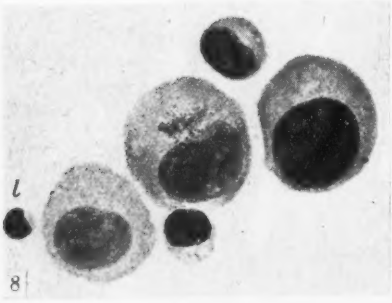
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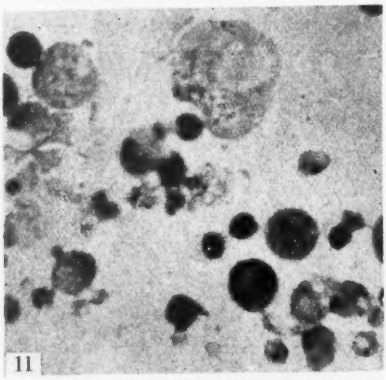
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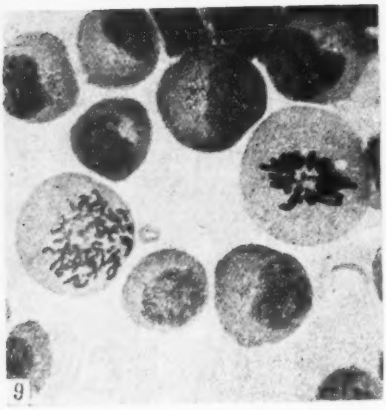
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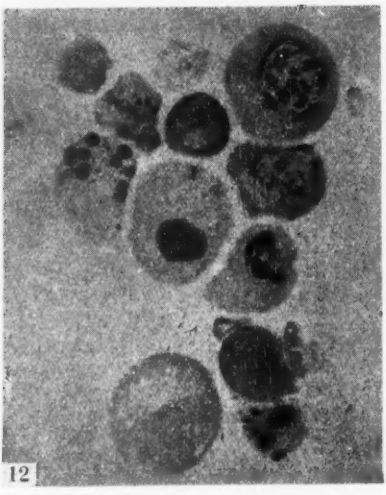
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要　旨

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まず「液態腫瘍」に關する著者の見解を述べた。それによると通常の動物の移植性の癌腫或いは肉腫も，例えば，それをエムルジオンの狀態にして腹膜腔内移植を反復することによって，液態腫瘍に變化せしめ得るだろうと豫想される。これに關する著者等の實驗は未だ成功していないが，累代移植性の液態腫瘍が得られれば，その形態學的研究はすべて塗抹標本で行うことが出來るから，形態學的研究に新生面を開くであろうし，腫瘍の生化學的方面の研究または化學療法の研究等にも多大の利便を與えるであろう。

吉田肉腫はこの意味の液態腫瘍の性質を完全に備えている。これはある發癌實驗を行っていた一群のシロネズミ中の一頭に發生したものである。その實驗で我々はこの種の腫瘍の發生を豫想していたのではなかったのであるが，上述の液態腫瘍の立場からこの特有な腫瘍に注目してこれを捉え，1943 年以來今日まで移植を續けている。移植は腫瘍腹水の一滴を他の動物の腹膜腔内に入れればよいので，甚だ簡單である。移植率は非常に高く 98 %である。動物は約 12 日の平均壽命で必ず死ぬ。その時は多量の腹水を生ずる許りでなく，組織内に廣く浸潤を生じ，その狀態は一般の惡性腫瘍と少しも變らない。腹水は 1 cmm 中に約 100 萬の腫瘍細胞を含んでいるから，細胞の檢査には遠心等の必要は全くない。卽ち血液檢査と全く同じ方式で腫瘍の檢査が出來る。

細胞は非常に大きく，正常の如何なる細胞よりも大きい。原形質内に多量のアズール顆粒を生ずることが一つの特徵である。細胞の性質は全體として單球に近い。

顯微鏡下で細胞の數をかぞえながら行った移植試驗で，唯 1 箇の細胞による實驗が成功した。30 箇以上の細胞があれば移植は常に確實である。すなわち，細胞があれば 1 箇でも移植し得るが，全く無細胞では植わらない。我々の今日までの研究の結果では，この腫瘍はウィールスによるものではない。少くとも，Rous の腫瘍または Shope の腫瘍等に知られているような意味のウィールスは證明出來ない。

今日までに我々は 45 種以上の化學物質をもって，この腫瘍で化學療法の基礎試驗を行ってみた。未だ完全に有效な物質には遭遇しないが，物質投與後の腫瘍の狀態を，逐時的に且つ細胞個別的に點檢することも出來るので，この腫瘍が，化學療法の研究に有效な試驗臺であることは示された。化學療法の研究はこれによって充分に組織立ったものになり得るだろうと期待する。今日までに最も效力の著しかったのは，チフス菌及び赤痢菌の培養濾液で，この濾液を腫瘍腹腔内に直接に與えると，約 10 日間の處置で，半數以上の動物が完全に治癒した。

[GANN, Vol. 40; March, 1949]

ON THE CORRELATION BETWEEN HISTOLOGICAL TYPES OF SQUAMOUS CELL CANCERS AND THEIR RADIOSENSITIVITY*

(Studies on cervix cancers)

(With Plate III)

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PREFACE

Duval and Lacassagne have noted that squamous cell cancers could be classified into at least two types: type cutanée (epidermoid type) and type muqueuse (mucodermoid type). A similar classification was given by Kortweg concerning cancers of the tongue: cornifying squamous cell cancer and solidum-type.

As is ususally the case these classifications were utilized as basis of prognosis, clinically and therapeutically. From Zuerich many works have been issued on this account, especially in relation to the radiosensitivity of a certain type of cancer. Thus Zwingli has classified some 500 cancers of the oral cavity into three main types: "Haut-" (epidermoid-), "Schleimhaut-" (mucodermoid-) and "Intermediär-" (intermediary-) type, among which special radiosensitiveness of mucodermoid type was said to be testified. In Japan Ban et al. have approved the work of Zwingli concerning cancers of the tongue and oral cavity. Ban on the same occasion made public a plan of classification of squamous cell cancers from his purely morphological point of view.

Recently as the radiotherapeutic measure of the cervix cancer of the uterus excells surgical one, there has arisen a demand from the clinical side to determine if there exists similar correlation between histological types and radiosensitivity of squamous cell cancer of the cervix uteri. On this account a few studies have been made but none of them has reached a definite conclusion. The same can be said on the relation of curability and histological malignancy referring to cervix cancers.

This study was at first schemed to find out some histological prototypes of cervix cancer if possible, which are specifically radiosensitive. It must be emphasized here that I have no intention of discussing radiocurability or histological prognosis of cancers but will deal with local radiosensitivity of them.

* This study was supported by the Research Funds of the Ministry of Education

Materials and Methods

A series of over 500 biopsy specimens from some 100 primary cancers of cervix uteri were subjected to a precise histological study before, during and after irradiation.

Irradiation was performed stereotypically: five radium-needles put in a prothesis (Pb. 1 mm: distance 0.5 cm), each needle containing 10 mg radium (Pt. 1 mm) placed intracavitally and two 10 mg needles inserted into the cervical canal; duration 23 hrs for once, interval 6 days; this was repeated generally 4 times as one course of treatment. In total 6,440 mg hrs were given in one course. (Forsell and Heyman's method modified by Yamakawa and Tsukamoto).

A biopsy specimen was taken from the central part of the cancer each time directly before the radiation, fixed in Maximow's fixative, cut in 3 microns sections, stained with H-E and Azan.

The original size of tumor before the therapy was classified in four grades; A, B, C, and D. The radiosensitivity was given purely clinically, according to the number of days elapsed before the tumor totally disappeared, in five gradings: 0, 1, 2, 3, and 4, as was done by Zwingli.

On Classification or the Squamous Cell Cancers

The first step to achieve the aim of this study may be to get a suitable plan of classification of cancers to be examined. The most detailed one ever made public is that of Ban which was fundamentally based on the idea of Duval and Lacassagne. I have at first expected to find a certain relation between the histological type of a cancer and radiosensitivity of it as Zwingli and Ban et al. obtained concerning squamous cell cancers of other organs.

But this attempt met with a great difficulty, as each of the fundamental criteria in the differentiation of epidermoid and mucodermoid type was very often found in the other type (Fig. δ' 3 and 4). They do not go parallel in indicating the types. Accordingly the classification was liable to be forced and subjective.

Zwingli himself already noticed that difficulty. It is natural that his intermediate type grew up to a bulky group of cases outnumbering the mucodermoid type. But, although the development of this mode of classification is a most conventional one, we should accept it, if they really have definite correlation to radiosensitivity of the cancers. On the contrary I could not find any possible correlation between them in case of cervix cancers classified somewhat forcibly after the manner of the French authors. (c-1, 2, 3-α, τ, δ in my classification

all roughly in the mucosal type and c-1, 2, 3-β, γ in cutaneous type) (Table 1) (Fig. 1, 3 and 4).

Moreover the fundamental criteria offered by Duval and Lacassagne seem to me not reasonable for the precise characterization of mucosal and cutaneous squamous epithelium in physiological conditions. They attempted to put forcibly too detailed an analogy between a normo-typical tissue and a cancer tissue, which might be regarded as being in various stages of anaplasia.

My own classification is characterized by an absolutely purely morphological point of view. All nomenclatures suggesting origin of the cancer cells were abandoned. For example the term "basal" in 'basal cell cancer or carcinoma basocellulare' was avoided, because a basal cell in a cancer indicates nothing particular than a spindle-shaped cell and the histological diagnosis of a basal cell cancer would be liable to be given to too many sub-types of tumors with variegated structures (at least to b-1, b-2 and some of c-1 in my classification) (Table 1. Fig. 2).

Even the term "squamous cell cancer" or "Plattenepithelkrebs" seems to me too artificial. Such cancers classified hitherto to this category may arise from a very extensive range of epithelium: squamous, transitionary, stratified cylindric and single-layered cylindric epithelium. Anyone who has some knowledge of Patzelt's work on the development of epiglottis epithelium can realize how much character a stratified squamous epithelium and a stratified cylindric epithelium have in common under physiological conditions. It might be more reasonable not to call a spindlo-polygonocellular carcinoma a squamous cell cancer. A term stratified cell cancer is offered here instead.

All solid cancers examined in this study were classified as follows:

Table 1

a. Carcinoma simplex: including types having no polarity in cancer cells.
   Carcinoma simplex macroalveolare a-1
   Carcinoma simplex microalveolare a-2

b. Carcinoma stratum non-differentiatum: including types having only a trace of polarity or frustrated differentiation to carcinoma stratum proper.
   Ca. stratum non-diff. spindlocellulare b-1
   Ca. stratum non.diff. polygonocellulare b-2

c. Carcinoma stratum proper: including types having definite tendency to differentiate into certain types of stratified epithelium, i. e. having two or more stages, each imitating a layer of stratified epithelium.
   Carcinoma stratum spindlo-polygonocellulare c-1
   Carcinoma stratum spindlo-polygono-lucidocellulare c-2
   Carcinoma stratum polygono-lucidocellulare c-3

simplex	c-1, 2, 3-α
spinosum	c-1, 2, 3-β
vacuolatum	c-1, 2, 3-γ
squamosum	c-1, 2, 3-δ
bulbiformicum	c-1, 2, 3-ε
keratosum	c-1, 2, 3-ζ

d. Carcinoma stratum mixtum: including types having more than one type above mentioned combined. d-o

Any qualifying adjunct may be given in addition, for example as to the mode of growth (endophyticum, exoxhyticum), pleomorphism (polymorphocellulare), special structures, etc., as Ban has introduced.

Another fundamental consideration should be made as to the classification of cancers, particularly as to whether a cancer tissue has a fixed type or not.

It is useless to-day to consider a cancer to be a fixed mature tissue when a cancer can be diagnosed from a single cancer cell. A cancer tissue consists of cancer cells, every one of which might be considered as a mutant from a normotypical epithelial cell. Cancerization means giving birth to a mutant which can develop autonomously by itself with aggressive character to the host tissue. In spite of our ignorance of the true cancerizing factor in general, there may exist enough reason to consider that certain cancerizing conditions can persist even after a cancer has developed. Even if it may not be the case, the mutants already produced would possibly continue to give birth to further mutants of any type, one of which with lethal factor might die away and the others with more adaptability could develop to some extent in loco. Many of them might fall in degeneration in turn without making an active focus of cancer. The fittest mutant or group of mutants which are provided with both intrinsic and extrinsic conditions or which can induce proper stromal reaction would be permitted to develop to an aggressive cancer. Formation of new mutants may be continuously repeated in the whole career of a cancer.

I have noticed in my series of the stratified cancer of the cervix some nests of cancer cells which have larger nuclei than normal producing buds of new type cell groups with markedly small nuclei. For example, in R-1296 the substantial part of the cancer is made up of carcinoma stratum (spindlo-) polygonocellulare with dominant size of nuclei of v=963 and with very slight endophytic tendency (type plicée). Buds of new nests are developing from the basal layer with much endophytic trend, characterized as carcinoma stratum non-differentiatum spindlocellulare with the dominant size of nuclei of v=1870 (Fig. 6).

In this way there may develop more than one species (or variant) of cancer cells in a cancer of an organ. The fact makes it difficult in a more detailed study of a cancer, theoretically and practically, to define it in one fixed type of cancer and to relate this typification with its radiosensitivity. I found that

each cancer cell nest may be different as to its type, consequently a section from a biopsy specimen may be labelled with several type specifications.

Why then for the majority of cancers a certain fixed classification may be permissible? To answer it is to assume that under given local conditions only one type or a few types of cancer could develop far more favorably than other possible mutants.

On Radiosensitivity and Histological Findings

Not a few studies have been made to find parallelism between the grade of anaplasia of cervix cancer and its malignancy or prognosis on curability.

Martzloff, MacCarty, Hueper, Warren and others have established each his own histological malignogram substantially on account of the grade of anaplasia in combination with other factors. Plaut and also Gulik found no correlation between histological malignogram of Martzloff and clinical and anatomical malignancy. But Warren and Otani et al. were affirmative to a more simplified classification of Broders in relation to anatomical malignancy.

I can not discuss here in detail on this matter, but, excluding grossly-topographic factors, there seems to exist a fundamental weak point in the idea of histological malignogram in dealing with a cancer as a fixedly established prototype of malignancy.

Gulik could not find any validity in Martzloff's malignogram in correlation with radiosensitivity. But Borak's "absolutely immature form of cancers" was said to be more radiosensitive and Béclère discussed parallelism of "dégrès décroissants" with radiosensitiveness of a cancer. MacCarty said on the contrary that combination of factors were more important than any single factor, including differentiation.

The results obtained from my study indicate 1) that a carcinoma simplex is not more radiosensitive than any other type of carcinoma stratum. They may possibly include not only the most anaplastic mutants of squamous epithelial cells but also other mutants originating from cylindric epithelium which is notably radioresistant. 2) Carcinoma stratum non-differentiatum is found to be more sensitive to radiation than more differentiated types of cancer. It might be seen as immature type of carcinoma stratum proper in general as the nomenclature indicates. 3) In the groups of carcinoma stratum of more differentiated character it cannot be recognized that the more the spindle-shaped cells predominate in the components of a cancer cell nest the more sensitive is the cancer as a whole to radio-energy (Table 2).

Different subtypes according to the cellular morphology of predominant cells do not necessarily represent grades of differentiation analogous to the cellular layers of normotypical squamous epithelium. There can be false differentiation

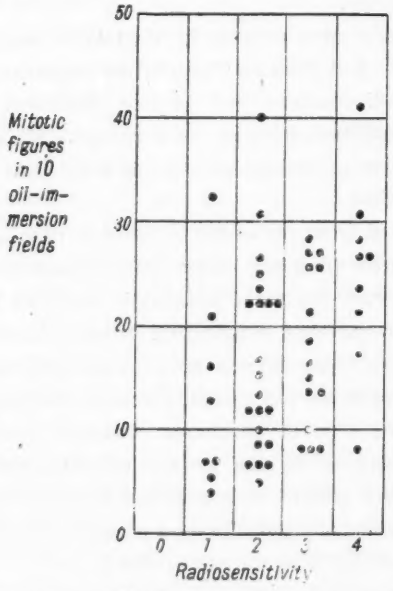
showing only outward resemblance.

It may be pointed out that in many cancer nests distribution of mitotic figures

Table 2. Histological types and Radiosensitivity

Histological types	Radiosensitivity					Total
	0	1	2	3	4	
Carcinoma simplex	0	0	1	2	0	3
Carcinoma stratum non-diff. spindlocellulare	0	2	0	4	3	9
Carcinoma stratum non-diff. polygonocellulare	0	0	1	4	1	6
Ca. strat. spindlo-(polygono)-cellulare simplex	0	0	5	4	2	11
Ca. strat. spindlo-polygono-cellulare simplex	0	1	7	7	3	18
Ca. strat. (spindlo-)polygonocellulare simplex	0	1	4	3	2	10
Ca. strat. spindlo-polygonocellulare spinosum	0	2	7	4	3	16
Ca. strat. spindlo-polygonocellulare keratosum	0	1	2	2	2	7
Ca. strat. spindlo-polygono-lucidocellulare	0	2	8	7	3	20
Total	0	9	35	37	19	100

Table 3. Number of mitotic figures and radiosensitivity.



Mitotic figures in relatively small cervix cancers (size-group B, C and D) were counted (oil immersion x 90; ocular x 10). Counts in 10 fields were added. Note the absence of correlation between number of mitotic figures and radiosensitivity.

Table 4. Distribution of mitotic figures in cancer cell nests and radiosensitivity.

	Radiosensitivity				
	0	1	2	3	4
Cancers with mitotic figures distributed all over the cancer cell nests.	0	1	0	3	5
Cancers with mitotic figures distributed in basal twothirds of the cancer cell nests.	0	2	4	2	1
Cancers with mitotic figures found only in several basal layers.	0	9	11	5	1

is fairly irregular: it can be limited to a few basal layers or can be distributed far into the central parts of the nest. Cancers with the latter type of distribution of mitotic figures show special sensitiveness to radiotherapy because a larger part of cancer cells might be destroyed by radiation which surely affect cells in division. For the same reason a cancer may appear to become much more differentiated after undergoing radiotherapy, as the more basally situated undifferentiated cells with nuclear division would be destroyed selectively and the more radio-insensitive central parts fall in swelling degeneration (Table 3 and 4).

To a certain extent radioeffect on the cancer depends upon stromal factors and this may be observed more exactly after a suitable dose of radiation was given than under untreated conditions. As is known the most notable stromal reactions are disturbances of circulation, some of which generally appear prior to cellular changes: edema, hemorrhage, cellular infiltration, dilation of blood vessels and degeneration of the walls of blood vessels. The radiosensitiveness of a tumor seems to go to some extent parallel with the intensity of these circulatory disturbances. Radioeffect on a living tissue, especially on cancer tissues, might well be reconsidered in reference to this phenomenon which might have certain connection with the idea of dysoria (Schuermann and M c Mahon) assuming toxic activity of blood components on the tissue.

Schmitz and Schmitz have recently noted characteristic degeneration of the polyhedral cells in the squamous cell cancers of cervix, cytoplasmic basophilia or acidophilia respectively after receiving radioeffects. Such tinctorial changes were also observed in the series of cancer in my study but a relation neither to the type nor to grade of radiosensitivity of the cancer was noticed.

Summary

1. On the radiosensitivity and histological findings:

a. A histological prototype with special radiosensitivity was not found in the serial study of cervix cancers.

b. Non-differentiated type of stratified cancer of the cervix is more sensible to radiation then more differentiated types.

c. Mere abundance of mitotic figures in the basal layers of cancer cell nests does not mean radiosensitiveness of the tumor as a whole.

d. Histological types with more widely and centropetally distributed mitotic figures in cancer cell nests are relatively sensitive.

e. A new idea to determine the radiosensitivity of a cancer according to parenchymal and stromal reaction after the first irradiation was discussed.

2. On the classification of "squamous cell cancer" in general:

a. Necessity of unprejudiced classification is stressed.

b. A new, purely morphological schema of classification and nomenclature is introduced.

c. The classification of stratified cancers into epidermoid and mucodermoid types which Duval and Lacassagne introduced and which Zwingli and Ban et al. have accepted as adequate to find correlation between histological types and radiosensitivity of the cancers in oral cavity was found not valid in application to the solid cancers of the cervix.

d. The criteria of Duval and Lacassagne's classification were discussed as not reasonable.

References

Ban, T.: Gann, 37 (1943).
Ban, T.: Gann, 37 (1943).
Duval et Lacassagne: Arch. franc. de pathologie gén. et expér. (1922).
Kortweg: Virchow's Archiv, 279 (1931).
Zwingli, F.: Z. f. Krebsf., 49 (1939).
Patzelt, V.: Z. f. Anatom. u. Entwickl.—geschichte, 70 (1924).
Martzloff, K.: Johns Hopkins Hosp. Bull., 34 (1923).
MacCarty, W. C.: Amer. J. Roentg., 37 (1937).
Hueper, W.: Arch. of Path., 6 (1928).
Warren, S.: Arch. of Path., 12 (1931).
Plaut, A.: Zbl. Gyn., 50 (1926).
Gulik, P.: Acta radiol. (Stockholm), 20 (1939).
Borak, J.: Strahlentherapie, 44 (1932).
Béclére: Acta radiolog, 6 (1926).
Schuermann and Mac Mahon: Virchow's Archiv, 291 (1933).
Schmitz, H., Schmitz, H-E., and Scheehan, F.; J.A.M.A., 112 (1939).
Krompecher, E.; Z. f. Krebsf., 19 (1923).
Lahm, W.; Strahlentherapie, 27 (1928).
Tsukamoto, K., and Oota, K.; Jap. Assoc. of Radiologist, 1948.

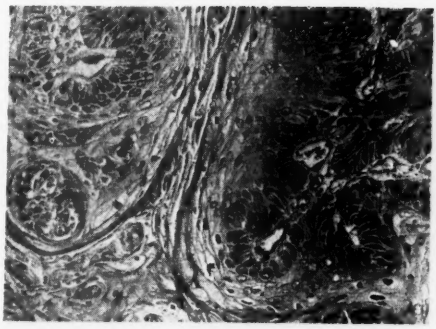


Fig. 1

Figure 1. (R-1184). Cervix cancer. Carcinoma stratum spindlopolygono-lucidocellulare spinosum keratosum. Distictness of intercellular spaces and cytoplasmic bridges in the basal layer and small polyhedral cell area and also parakeratotic cornification indicate epidermoid type of Duval and Lacassagne, but the compactness of large clear polyhedral cells is on the contrary characteristic of mucodermoid type of the authors. (Zenker, H-E., x 160)

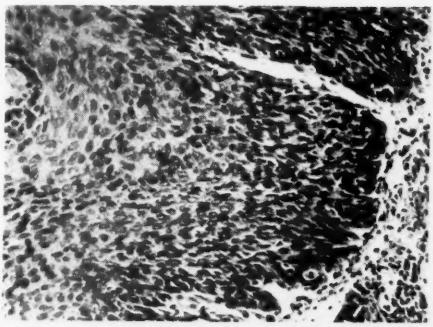


Fig. 2

Figure 2. (R-1223). Cervix cancer. Carcinoma stratum spindlo-polygonocellulare partim squamosum. Note distribution of mitotic figures not only in the basal spindle cell layers but also in the polyhedral cell layers at the left. Although some would classify this type of cancer to so-called basal cell cancer there exist distinct differentiation of the cells from spindle cell type to polyhedral cell type. The extreme left part shows desquamative change. (Zenker, H-E., x 210)

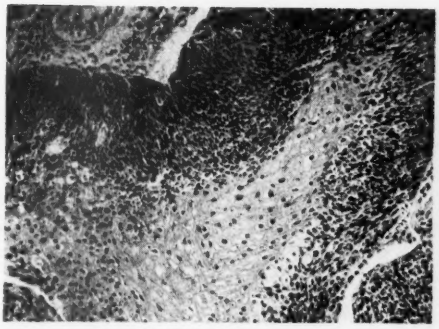


Fig. 3

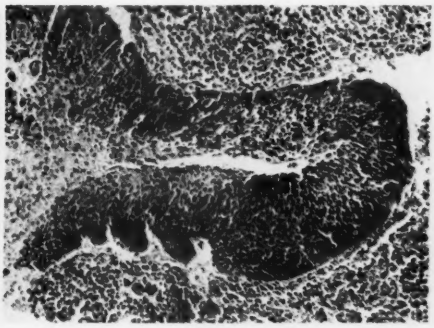


Fig. 4

Figures 3 & 4. (R-1330). Cervix cancer. The former is carcinoma stratum spindlo-polygono-lucidocellulare spinosum bulbiformicum belonging to epidermoid type of Duval and Lacassagne and the latter is carcinoma stratum spindlo-polygonocellulare squamosum belonging to "typical mucodermoid type". These two types are found side by side in the same preparation (Zenker, H-E., x 160).

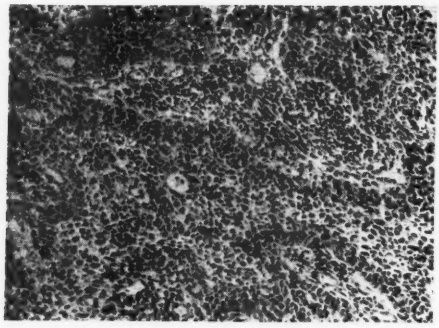


Fig. 5

Figure 5. (R–1369). Cervix cancer. Carcinoma stratum nondifferentiatum polygonocellulare polymorphocellulare. This cancer is fairly anaplastic but should not be classified to carcinoma simplex because certain trends to imitate stratified epithelium are noticeable (Zenker, H–E., x 160).

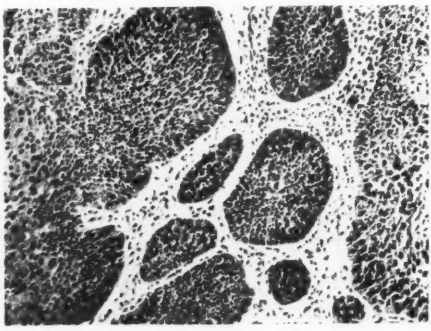


Fig. 6 b a

Figure 6. (R–1296). Cervix cancer. Carcinoma spindlo-polygonocellulare. Note remarkable differences in size of nuclei of the basal cells in part (a) and (b). Average size of nuclei in (a) 963 cubic microns, in (b) 187 cubic microns. Note also the remarkable endophytic character of (b) forming rather small cell nests. The part (a) has rather surface-covering trends.

要　旨

扁平上皮癌の組織像とその放射感受性の相關について

（子宮頸部癌の研究）

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約 100 例の子宮頸部癌を，Ra- 照射治療の前，照射間及び後に，連續的に組織學的檢査を施して，その組織像と放射感受性について，相關を求める目的をもって研究した。

1. 從來のいわゆる扁平上皮癌なるものの分類に對して，純粹に形態學的の立場から新分類を提出した。殊に扁平上皮癌に對しては，重層上皮癌の新命稱を提唱し，且つ Duval 及び Lacassagne のいわゆる粘皮型及び表皮型なる分類に批判を加えた。

2. 特異的に放射感受性の高度の組織型は見出し得なかったが，未分化型重層上皮癌は分化型並に單純癌に比して，感受性の高いことを認めた。

3. 有絲核分裂像の多少は必ずしも放射感受性の強弱と併行しないが，一癌細胞巢内において，核分裂像がその中心部にまでも廣く分布している場合には，明らかに高い感受性が見られる。

4. 放射感受性を檢索する一つの着眼として，第一回照射後における組織像の變化，殊に，癌巢と間質との交涉を檢索する必要のあることが強調せられる。

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STUDIES ON STROMAL REACTIONS OF THE REGIONAL LYMPH-NODES IN CANCER

(With Plate IV)

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PREFACE

Now that a cancer can be diagnozed from a single cancer cell in smear preparatation, as demonstrated by Papanicolaou, it seems to be reasonable that the idea of cancer as an entity consisting of groups of cancer cells and stroma should be modified somehow. The weight of stroma in cancer problem has been much reduced. For all that the stroma of cancer must be recognized as a zone of interrelation between cancer cells and host tissue. It is obvious that the stroma of cancer is generally produced from host tissue. What then really induces the host tissue to produce the stroma and how may this occur?

In order to answer these questions it may be convenient to pick out the two extremes among many types of cancer from the point of view of the content of of stroma: medullary and scirrhous cancers. The abundance of stroma in the latter might be caused either (1) by factors concerning natures of the cancer cells, (2) by factors concerning changes in the characters of host tissues, or (3) by factors casually influencing both or either of them, for example, infection existing or having exited in the host tissue.

Many studies on the structure of primary foci of cancers have yielded so far not much for the solution of this problem, either because the factors concerning host tissues are generally too complicated owing to the multiple components in the elementary structure of the tissue, especially to the existence of potent mesenchymal tissues in the perivasculary spaces, or because the primary focus is very liable to infections after formation of an ulcer or necrosis.

We have found that a study on lymph-nodes of the regional lymphatic system belonging to a cancer lesion might well exclude, to a certain extent, those difficulties inevitably connected with the studies on primary focus. The lymph-nodes have relatively simple composition, especially in regard to its interstitium which is built chiefly of reticulo-endothelial elements and have limited numbers of entrances for cancer cells and any other elements coming in. They also receive limited inflammatory irritation form the causative agent of infection and, in addition, as they gain distance from the primary focus, the inflammatory

effects become markedly weak in intensity.

On the other hand we should be concious of the fact that a lymph-node has no detailed general pattern in interstitial construction which may vary in wider range accoring to the history of the organ and the person as a whole, for example to age, constitution (Orsos) and general or localized pathological process. But, taking suitably located lymph-nodes of the same person or adequate parts of the same lymph-node for comparison, we can to a certain extent eliminate the difficulties due to those miscellaneous influences on the organ in getting comprehensive findings or the changes in certain parts of a lymph-node induced by the presence of cancer in the primary focus.

Materials and Methods

In all 90 stomachs, surgically resected because of gastric cancers, were histologically examined. All the lymph-nodes resected with the stomachs were checked as to their locations and were submitted to detailed histological examination. H-E, Mallory and Dublin's silver stain were applied. Each stomach has some 15 to 30 lymph-nodes with it and so over 1000 lymph-nodes were studied. Primary focus and lymph-nodes with or without metastasis were subjected to histological comparison. Elastic fibres (s. Orsos) were not stained.

Findings on Primary Foci

The primary tumor are classified exclusively according to the relative content of fibrous elements as follows:

Groups	parenchyme	stroma	Number of cases
I	+++	+	28
II	++	++	37 (* 4)
III	+	+++	26

Group I includes all the very medullary cancers, group III the scirrhous ones. The majority of group II has much parenchyme and moderate quantity of stroma, and accordingly is rather medullary. Group II includes also partly sirrhous and partly medullary cancers*.

The classification is rather conventional, for a very medullary cancer may grow in part in a densely fibrous stroma, especially in its peripheral parts, and a scirrhous cancer may grow almost invariably in very medullary type in mucosa, the cancer cells presenting themselves as signet ring cells.

All very medullary cancers fall without exception in necrosis in their central parts and build deep ulcers. Ulcerations in the scirrhous cancers of the stomachs were classified as follows;

Scirrhous without ulceration:	3	
Scirrhous with ulcerations:	23	
Shallow ulceration of limited size		12
Shallow ulceration, extensive		4
Deep ulceration of limited size		3
Deep ulceration, extensive		4

Findings on Regional Lymph-Nodes

1. Cellular reactions in general of the regional lymph-nodes

The cellular reactions of regional lymp-nodes in case of cancer have been repeatedly reported. Our findings on this account differ not much from those already reported by others.

All the lymph-nodes examined showed at least some changes. General atrophy is said to be common to old age, but in our materials, ranging from 30 to 70 years of age, about two-thirds of the lymp-nodes were not atrophic nor apparently sclerotic as was advocated by Roessle and Yoshida. Acute or subacute purulent inflammatory reactions, which were found very rarely, were limited to those cases with very remarkable purulent inflammation in the primary foci following necrosis of medullary cancers. We found them in no case of a scirrhous cancer. Endothelial hyperplasia and sinus catarrh in more localized or diffuse type, and generalized or more or less circumscribed hypertrophy of reticulum cells were found very common combined with varying grades of phagocytosis and hemosiderosis or erythrophagia. Various types of germ-centres were seen, among which R-type of Ono and Nakagawa was rather common. Hyperplasia of lymphocyts, eosinophilic reactions and hyalinous deposition were very rarely seen.

These cellular reactions seem to be very irregular in incidence: they can be very conspicuous in one node and entirely lacking in another. Various types of reactions can occur in a series of lymph-nodes in the same person interchangingly. So we could not get a definite difference as to the types and intensity of cellular reaction in the lymph-nodes, between medullary and scirrhous cancers. Also it cannot be said that they form a discrete pre-invasive cellular reaction as is proposed by Guttner and others.

2. Stromal reaction in lymph-nodes with metastasis

In general there can be found a thrombus of cancer cells in one or several vasa afferentia before a sinus-metastasis is detected. Here exists neither cellular nor fibrilloplastic reaction in the wall. On no occasion any kind of organizing change from intima was found. They may develop thence forward to build up periglandular metastasis, which in general form a very fibrous type of growth independent of the original characteristics of the primary cancer.

The most common type of lymphadenic metastasis of a cancer is that in the marginal sinus.

A few cancer cells may show themselves mixed up with swollen sinal reticulum cells (Akazaki and Murakami), where sometimes it is very difficult to differentiate

a tumor cell from host cells, because they show every type of regressive changes involving both nucleus and cytoplasm. Possibly they may be eliminated there or may survive in the stationary state for some time. Fibrillar structures present no particular changes in this stage.

As they develop they make either a nodule or a diffuse endosinal extension. In case of medullary cancer the delicate reticulum-fibrils of the sinus will be invariably amputated, may it be caused by distorsion under compression of the tumor cells or by dissolution through unknown mechanism. Also in case of scirrhous cancer the sinus reticulum can be rarified in the same manner, but about half of the lymph-nodes examined in the regional system of scirrhous cancers showed definite hypertrophy or hyperplasia of reticulum-fibrils in the developing cancer metastasis. This may extend to a certain distance in the neighboring part of the marginal sinus remote from the actual site of cancer. There may exist some lymphocytes in the sinus but no leucocyt.

A metastatic part of a scirrhous cancer in a lymph-node does not evoke generalized sclerosis in the whole node ; the fibrillosis mentioned seems to remain, as a rule, localized in an area directly in contact with the metastasis or in more or less wider portion of the lymph-node irrigated by lymph sap from the focus. The rest of the lymph-node remains intact so far as the fibrous elements are concerned.

A denser net of fibrils may be built around the nodule, which in turn may be dissolved in case of a medullary cancer and thickened in case of a scirrhous cancer.

As a scirrhous cancer develops over the thick fibrillar membrane marking lymphatic follicle from the sinus, this hyperplastic process of fibrils is especially remarkable. Both horizontal and perpendicular fibrils thicken themselves. In addition obliquous or irregularly running fibrils are liable to be built exactly on the spot, in the front of a growing cancer nodule.

However scirrhous cancer does not necessarily build a very fibrous nodule. In certain case a very medullary metastatic nodule, which definitely dissolves fibrillous stroma, may be given birth in a lymph-node, where other cellular reactions are prevailing in the same mode as in the other ones with or without fibrous metastasis.

The fibrous reaction of lymphadenic tissue against a medullary cancer begins with apparent thickening of reticular fibrils displaced by a nodule ; this however will be dissolved as soon as the metastatic nodule grows progressively. A true fibrillosis may be launched after the nodule has reached its stationary phase somehow. Degenerative changes in the nodule decrease exserting pressure on surrounding fibrils, and introduce organizing process from the surrounding tissue, which result in definite fibrillosis and fibrosis in and around the metastatic nodule of a medullary cancer.

3. On the pre-invasive fibrillosis

The findings mentioned above and those in the peripheral zones of a scirrhous cancer, especially of mammary gland, suggest that a kind of fibrillous reaction might precede, to a certain extent, actual invasion of tumor cells. It has led us to the assumption that there may actually exist a fibrillosis in lymph-nodes, regional to a scirrhous cancer.

In the series of 26 cases of scirrhous cancers of stomach, we could really find out such fibrillosis in 16 lymph-nodes of 6 cases. These correspond to 23 % of all the scirrhous cancer cases examined, 13.6 % of all lymph-nodes examined in scirrhous cancer cases and 28 % of lymph-nodes without metastatic changes among them.

At one pole or along one side of a lymph-node, mainly facing to the primary focus, the perpendicular fibrils or networks of fibrils of the marginal sinus, which is often enough moderately dilated, are definitely strengthened. Collagenization of the argyrophilic fibrils or definite fibrosis is not a common appearance. Cellular reactions differ much in each case and have not definitive relation to the fibrillous change. Hypertrophic or hyperplastic process of reticulum cells in the marginal sinus does not necessarily mean hypertrophic or hyperplastic changes in argyrophilic fibrils.

Inflammatory influences should be excluded in order to attribute this to a scirrhous character of the tumor in the primary focus. In all cases having "pre-invasive fibrillosis," an active inflammation was absent both in the sinuses andin follicules of the lymph-nodes. Shallow and inextensive ulceration in the primary focus of scirrhous cancer in comparison with deep and extensive ulceration common in medullary cancers, together with common inflammation of rather limited intensity and extent in the primary focus of scirrhous cancer might probably support our view to attribute this fibrillosis to the specific character of scirrhous cancers. In addition we could not find any such fibrillosis in a series of regional lymph-nodes belonging to more or very medullary cancer of stomach.

4. On the cause of fibrosis in scirrhous cancer in general

What is really the causative agent in inducing such fibrous reaction in a scirrhous cancer? Several possibilities can be counted as follows:

a. Special trends in general on the part of the host.

b. Co-existing third factors, especially inflammatory ones.

c. Type-specific intrinsic factors on the part of cancer cells, for example secretion of special, fibrosis-inducing factor from cancer cells themselves.

d. Factors concerning rapidity of growth: all types of cancer cells can

induce a fibrous reaction to some extent, but a more rapidly growing cancers destroy the fibrous wall in making and a more slowly growing one may permit the full development of the wall.

e. Fibrogenic factors in connection with degenerative change in cancer: any cancer cell in the front of growth may undergo degeneration, which in turn somehow stimulate the host tissue to produce fibrous reaction in loco. A scirrhous cancer may invade into host tissues after much unsuccessful trials: thus the fibrous stroma of a scirrhous cancer may apparently be preformed.

Possibly we can exclude a. and b. without further consideration. If the pre-invasive fibrillosis in regional lymph-nodes could really be established, it might be said that we could also exclude d. and e. and attribute the fibrous character of a scirrhous cancer to an intrinsic factor of the cancer cells directly.

Summary

1. In case of endosinal metastasis of a medullary cancer argyrophilic fibrils will invariably disappear or will be weakened.

2. On the contrary an endosinal metastasis of a scirrhous cancer may give rise to a localized fibrillosis of marginal sinus, if not regularly.

3. Further development of cancer metastasis causes either fibrillosis or fibrillolysis in front of the growth, according to the nature of the primary cancer, scirrhous or medullary.

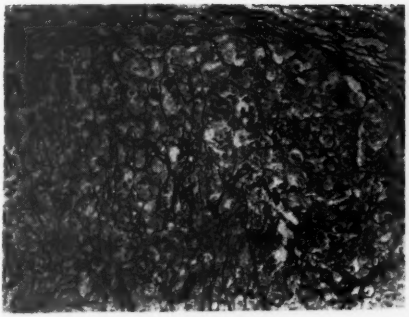
4. A cellular pre-invasive reaction in a regional lymph-node cannot be identified.

5. Possible pre-invasive fibrillosis in the marginal sinus in a series of regional lymph-nodes of scirrhous cancer was suggested.

6. Possibility of attributing fibroplastic trends in a scirrhous cancer to certain intrinsic factor of the cancer cells was discussed.

References

Orsos, A.: Ziegler's Beiträge 75 (1926).
Roessle and Yoshida: Zieglers Beträge 45 (1909).
Orsos, A.: Verh. dtsch. Path. Ges. 20 (1925).
Boehmig and Schroeder: Z. f. Krebsf. 48 (1938).
Bazzochi, G.: Clinica 4 (1938), Ref. Z. f. Krebsf. 48 (1938).
Buengeler, W.: Med. Welt 1938.
Borst, M.: Die Lehre von Geschwulst, P. 58-,
Schmidt, W.: Z. f. Krebsf. 48 (1938).
Guttner, H.: Z. f. Krebsf. 53 (1942).
Kuschfeldt, R.: Z. f. Krebsf. 46 (1937).
Akazaki and Murakami: Igaku to Seibutsugaku, 6 (1944).
Murakami, T.: Mitt. aus d. path. Inst. Niigata, Heft LXII (1945).



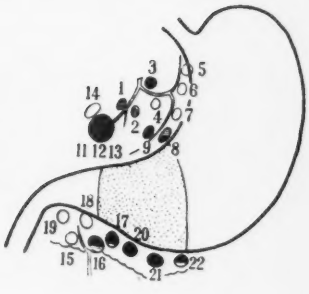
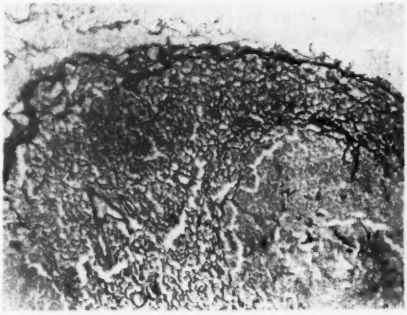


Fig. 1



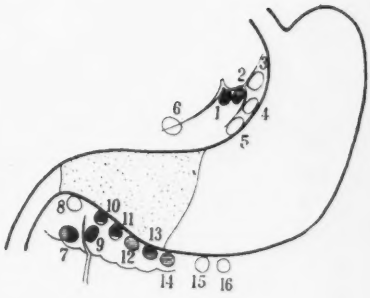
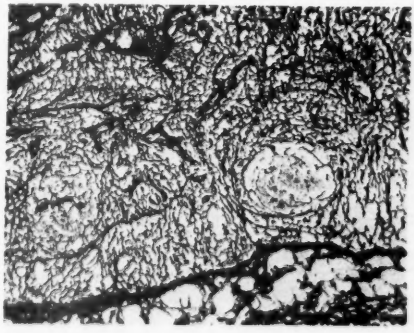


Fig. 2



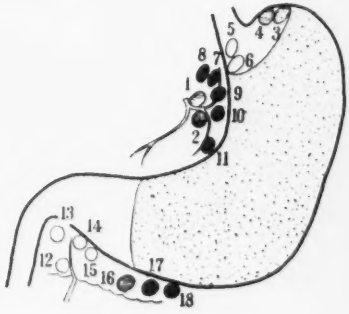


Fig. 3

Explanation of Plate IV

Figure 1. 0-146. L-13. Developed marginal sinus metastasis of scirrhous gastric cancer. Note hypertrophic argyrophilic fibrils in the compact cell nests. (Formol, Dublin's silver stain, x 240). Lymphadenic metastases were found in lymph-nodes 3, 8, 9, 11–13, 16, 17, 20, 21 and 22; pre-invasive fibrillosis was seen in 1, 2 and in part of 8. Ulcer in the primary focus was shallow and limited.

Figure 2. 0-253. L-7. Moderate pre-invasive fibrillosis of the marginal sinus remote from existing metastatic nodule in the same lymph-node regional to scirrhous gastric cancer. (Formol, Dublin's silver stain, x 140).
Lymphadenic metastases were found in lymph-nodes 1, 2, 7, 8, 9, 10, 11, and 13; preinvasive fibrillosis was noted in 12, 14 and parts of 7 and 13. The primary focus was not ulcerous.

Figure 3. 0-132, L-4. Remarkable pre-invasive fibrillosis of sinuses in a lymph-node regional to scirrhous gastric cancer. (Formol, Dublin's silver stain, x 140).
Lymphadenic metastases were found in lymph-nodes 2, 7, 8, 9, 10, 11, 17 and 18; pre-invasive fibrillosis was seen in 1, 3, 4and 16. The primary focus shows shallow limited ulceration.

要　旨

癌における局所リンパ節の間質性反應について

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（癌研究所）

目的　腫瘍の間質性反應を檢討するには，その原發巢を檢索しても分析が困難であるが，リンパ節轉移における態度はリンパ節なるものの間質の基本構造と反應樣式が單一であるために都合がよい。

この目的で殊に髓樣癌と硬性癌の局所リンパ節を比較檢討した。

材料・方法　91 例の胃癌切除例の胃について，その原發巢とリンパ腺（各例につき 15~30 個を比較檢討した。

染色　H-E 染色，Mallory 染色，Dublin 氏鍍銀染色

結論

A. 轉移初期には緣洞內轉移において

1）髓樣癌ではほとんど一樣に緣洞銀纖維は消失に向う。

2）硬性では緣洞銀纖維の肥大，新生が局在性に見られることがある。但し離斷消失の起る場合もある。

B. 轉移がさらに成長すると，成長の前線において

Fibrillosis——硬性

Fibrillolysis——髓樣癌

が進行する。

C. 轉移巢內の二次的退行變性は髓樣癌中にも Fibrillosis—Fibrosis を起さしめる。

D. Pre-invasive cellular reaction（侵襲前の細胞性の反應）として一定のものを認めることは出來ない。

E. 然し Pre-invasive fibrillosis と考えられるものが存在するように思われる。

發現率 26 例中 6 例（16 個のリンパ腺）

發現場所は圖表を參照

但しこれに對しては各リンパ腺の既往について對照がなければならない。同一個體の同一群に屬するリンパ節或いは同一リンパ節の他の部分との比較において，かなり炎症性要素を除外し得ると思う。

F. もし Pre-invasive fibrillosis があるとすれば，それは硬性細胞の fibroplastic intrinsic factor を考えねばならない。

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TOXOHORMONE: A CHARACTERISTIC TOXIC SUBSTANCE PRODUCED BY CANCER TISSUE

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INTRODUCTION

The idea that some sort of toxic substance may be produced by cancer tissue is of long standing, and is supported by the well recognized occurrence of cancer cachexia, frequently out of all proportion to the size of tumor. "As yet, however, it has been impossible to demonstrate any characteristic toxic substance in cancer," in spite of innumerable attempts in the past. The previous failures are probably due to the lack of appropriate criterion upon which to base the assay of such a substance.

In recent years, evidence has been accumulated, especially by Greenstein and his co-workers, closely associating the decrease of liver catalase with the growth of tumor, and, in fact, their experiments established that the decrease of liver catalase is one of the outstanding systemic changes in neoplastic diseases. Utilizing this fact as the starting point we succeeded in separating from cancer tissues a substance which is characterized by its ability, when injected into animals, to markedly decrease liver catalase. The substance can be clearly distinguished from other toxic constituents extractable from cancer tissues as well as from necrotic non-malignant tissues, these latter substances being highly toxic in that they kill animals in relatively small amounts and yet their maximum sublethal amounts scarcely affecting liver catalase.

In our preliminary report[1] we proposed a tentative designation of "toxohormone" for the substance which we separated. It is apparently produced by cancer cells, whence it is thrown into general circulation and acts to interfere with a certain specific normal function in the animal body. The term is intended to indicate a sort of internal secretion which, instead of stimulating, inhibits a normal cellular function.

THE INITIAL PROBLEM

It has long been known that not only tumor tissue itself but also organs, especially liver, of tumor bearing man and animals are markedly deficient in catalase activity [2,3]. Greenstein and associates [4~8] demonstrated the same fact in rats and mice bearing a wide variety of tumors, both transplanted and spontaneous,

the catalase decrease being progressive with and proportional to the growth rate of the tumor. They discovered an extremely significant fact that the extirpation of the tumor caused the liver catalase to rapidly return to normal, reimplantation with tumor causing liver catalase to drop again. They also found that the liver catalase activity of pregnant mice and of mice bearing large, progressively growing embryonic tissue implants was normal, showing that the effect on liver catalase is not due to any growing tissue within the body but it is specifically related to the fact that the growing tissue is malignant. By demonstrating that tumor tissue has no direct action *in vitro* on liver catalase, they concluded that the low liver catalase activity of tumor bearing animal is ascribable to the reduction in amount of total catalase present in liver.

A natural inference from these observations may be that tumor tissue produces some toxic substance which, acting upon liver cells, serves to decrease their catalase content. In the hands of Greenstein, however, tumor extract injected into normal rats produced no change in the liver catalase activity of these animals, which led him to conclude that "the living tumor in the animal body is a necessary condition to the effect which is produced on the liver catalase mechanism."

However, this failure of tumor extract to affect the liver catalase does not seem sufficient to reject the hypothesis that a toxic substance is given off by the tumor, for the toxic substance in question may occur in tumor tissue only in a low concentration and it may require a very large quantity of an ordinarily prepared extract to deliver a sufficient amount of the substance to affect the liver catalase activity *in vivo*. The crucial point seems to be whether the hypothetical toxic substance can be separated from cancer tissue in a sufficiently concentrated state to meet the experimental requirement, and this constituted the initial problem in the present investigation.

Experimental Method

In the present investigation we used human cancer tissues, mostly obtained at operation. Tissues were treated in various ways and each sample of various fractions was tested by injecting intraperitoneally into normal mice, in order to determine its liver catalase depressing action.

Approximately 20 hours after the injection the mice were killed by exsanguination and the liver removed, the 20 hour period being empirically chosen as suitable for the purpose.

The method for the determination of catalase activity was as follows:—A piece of liver tissue, weighing accurately 0.1 g, was ground up in a mortar and thoroughly extracted with 10 cc of phosphate buffer solution of pH 7, and centrifuged at 3,000 r. p. m. for 5 minutes. 0.3 cc of the extract so obtained was

diluted with 0.7 cc of the same phosphate buffer solution, and its catalase activity was measured gas-volumetrically with the aid of a Battelli-Stern apparatus at room temperature, the duration of the reaction being 5 minutes. The cc of oxygen obtained was recalculated into that at the standard condition.

PRELIMINARY DATA

Liver Catalase Activity of Normal Mice

In order to furnish a basis for comparison it is necessary first to present our data on liver catalase activity in normal mice, as determined by the above method. In Table 1 are given the liver catalase activity, expressed by the cc of oxygen, in 73 normal untreated mice. These include all the blank controls carried in conjunction with the various test series to be described later in this paper.

Table 1. Liver catalase activity of normal mice. (Oxygen cc.)

Mouse No.	Catalase Activity	Mouse No.	Catalase Activity	Mouse No.	Catalase Activity
1	16.4	26	8.5	51	6.3
2	13.0	27	8.3	52	6.3
3	12.8	28	8.3	53	6.2
4	11.6	29	8.1	54	6.2
5	11.5	30	8.1	55	6.2
6	11.5	31	8.1	56	6.2
7	11.0	32	8.0	57	5.9
8	10.9	33	8.0	58	5.9
9	10.7	34	7.9	59	5.8
10	10.5	35	7.8	60	5.8
11	10.0	36	7.8	61	5.7
12	9.9	37	7.7	62	5.7
13	9.8	38	7.7	63	5.6
14	9.7	39	7.6	64	5.4
15	9.3	40	7.4	65	5.4
16	9.2	41	7.3	66	5.3
17	9.1	42	7.3	67	5.3
18	9.0	43	7.3	68	5.2
19	9.0	44	7.3	69	5.1
20	9.0	45	7.1	70	5.0
21	8.9	46	6.9	71	4.9
22	8.8	47	6.8	72	4.7
23	8.8	48	6.7	73	4.6
24	8.8	49	6.5		
25	8.7	50	6.5	Average	7.87

The very wide range in the individual variation of liver catalase activity is notable among normal, untreated mice. It may be pointed out, however, that

no case of less than 4 cc oxygen was encountered and those below 5 cc amounted to only 4 percent of the total cases.

Liver Catalase Activity in Mice with Spontaneous Tumors

We have had the opportunity of making the liver catalase determination on four cases of spontaneous mouse cancer, exactly in the same manner as above. Tumors in these mice were all the usual mammary carcinoma and were of several weeks duration after their first detection, attaining fairly large sizes. Although the number of the mice investigated is very small, the figures obtained may be of interest in suggesting that the oxygen value of around 4 cc or less may safely be taken to reflect the liver catalase depressing effect of cancer tissue in mice.

Table 2. Livercatalase activity in mice with spontaneous cancer

Cancer mouse No.	Liver catalase activity (Oxygen cc)
1 (Albino, stock mouse)	4.3
2 (Dba strain)	4.1
3 (,, ,,)	4.1
4 (C3H strain)	3.9

SEPARATION OF ACTIVE FRACTION

Ether Extract: Preliminary experiments using gastric cancer tissue from three different cases clearly showed that the substance we have in mind is not ether soluble.

Ether extract was prepared from dried cancer tissue and was injected intraperitoneally into normal mice in 100 mg amounts (emulsified in distilled water with the aid of a little NaOH). This amount proved to be close to the minimum lethal dose, and one out of six injected mice died in several hours, while those surviving showing signs of general intoxication with diarrhoea. In spite of these facts the liver catalase activity was found not to deviate from normal: 13.9, 7.3, 6.7, 6.5, and 6.3 (average: 8.1).

Alcohol Filtrate: Cancer tissue either fresh or in the form of dried material was extracted with distilled water under heating in a boiling water bath for a few hours, after which it was allowed to stand overnight. Watery extract was concentrated to about 1/10 volume over hot water bath, and twice the volume of absolute alcohol was added and mixed, upon which voluminous precipitate formed. After standing overnight, the mixture was centrifuged, separating precipitate and filtrate, which were then dried and washed with ether.

Alcohol filtrate, or the extractive fraction, from three different cases were tested on 6 normal mice in 100 mg amounts, without showing any change in

the liver catalase activity, figures being 8.5, 7.5, 7.0, 6.4, 6.2 and 6.1 (average: 6.9). This fraction was readily soluble in water, giving clear brownish yellow solution.

Alcohol Precipitate (Active Fraction): The alcohol precipitates prepared in the above described manner from different samples of tumor tissue were tested by injecting mostly in 50 or 100 mg amounts.

The generally low figures for the liver catalase activity noted after the injection were in a striking contrast to all the preceding data, and clearly demonstrated that the alcohol precipitate carries the active principle which is capable of of reducing the liver catalase activity.

The objective manifestations which follow the injection of the alcoholic precipitate in the maximum amount (100 mg) used in these experiments were transitory discomfort and general debility, which disappeared usually by the next morning. In few exceptional cases the alcoholic precipitate was found to kill a mouse overnight in 100 mg doses, but no parallelism was noted between the toxicity and the liver catalase depressing action.

Experimental details with different tumor tissues may be given as fellows:—

Gastric Carcinoma

By far the largest proportion of our material consisted of gastric carcinomata, all surgical cases. In most cases the cancer tissues were dried in an open dish over boiling water bath before extraction with water, but on a few occasions extracts were obtained directly from fresh cancer tissues.

1. Dried cancer tissue was roughly comminuted and was extracted with distilled water which was added at the rate of 40 cc to 1 g. The mixture was well stirred and shaken under heating in boiling water bath for 1-2 hours, after which it was allowed to stand overnight either at room temperature or in ice box. Before extracting with water the dried material was washed with ether if it appeared to contain much fat. Watery extract was separated by centrifugation, evaporated to about 1/10 volume over hot water bath, and twice the volume of absolute alcohol was added and mixed, upon which voluminous whitish precipitate formed. During the concentration of the water extract varying amounts of coagulum appeared, which was not removed and which was consequently included in the precipitate formed by alcohol. After standing overnight in ice box the mixture was centrifuged, separating the precipitate, which was then dried and washed with ether.

The alcohol precipitate prepared in the above described manner was usuully light brownish in color. The yields varied from 6 to 10 percent of the original dried material. It contained much water insoluble matter, so it was necessary to reduce it into very fine powder before taking it up in distilled water for

injection, soluble and insoluble matters being evenly mixed and injected together.

Seven samples of alcoholic precipitates were tested, and all but two of them were found to reduce the liver catalase activity beyond the limits of the normal variation. Figures (cc of oxygen) are: 2.3, 4.6 and 4.8 at 25 mg and 2.8 and 3.3 at 50 mg amounts for TT, 3.0 and 3.3 for HD, 3.6, 4.2 and 4.6 for YS, 3.6 and 10.8 (!) for HT, all at 50 mg, and 2.4 and 2.8 at 100 mg amounts for a pooled sample from five cases, at 100 mg amounts. Of the remaining two samples, one (TH) gave low but "dubiously positive" figures (4.6 and 5.5), while the other (ID) failed to affect the liver catalase (6.4 and 7.8), both at 100 mg level.

As to this inactive sample (ID), it may be stated that in a later experiment 1.2 g of it was throughly extracted with 50 cc of distilled water under heating, yielding 1 g of water soluble and over 0.1 g of insoluble matters. When tested in 100 mg doses, the insoluble residue was inactive but the soluble fraction gave 5.6 and 3.8 values, showing that even from such an inactive precipitate as this the active principle can be recovered by re-extraction with water. Obviously the activity of the alcohol precipitate depends upon its water soluble matter. It seems likely that, had the coagulum formed during the concentration of water extract been removed before precipitating with alcohol, a more active precipitate might have been obtained in the first instance.

2. Three samples (KB, YB, and HD) of gastric cancer tissue were extracted without first drying them. The fresh tissue was comminuted, distilled water added at the rate of 200 cc per 50 g, well stirred and shaken, and heated in boiling water bath for 1–2 hours. After standing overnight, the mixture was centrifuged, the clear extract concentrated to a suitable volume over hot water bath, and precipitated by adding twice the volume of absolute alcohol. The coagulum formed during the concentration of the extract was not removed before adding alcohol. The alcohol precipitate was dried and washed with ether. Yield: 1–3 percent of the fresh tissue.

The precipitate from the three different sources were found to be active in depressing liver catalase activity in 100 mg amounts, the figures being 2.8 and 5.3 for KB, 3.3 and 7.9 for YB, and 3.7 and 4.2 for HD.

Liver Metastases of Gastric Carcinoma

This sample was obtained at autopsy. The case (MA) was gastric carcinoma with large metastases in the liver, and the metastatic growth alone was available for our purpose.

Dried material, 12 g, was extracted with water in the usual way, extract evaporated to 1/10 volume, all the precipitates, including the coagula formed during concentration, were contrifuged off, and alcohol precipitate was prepared

in the usual way from the centrifuged supernatant. Yield of alcohol precipitate: 350 mg.

This precipitate proved to be quite active, the liver catalase activity of mice injected with it in 50 mg doses being 2.8 and 2.7.

Rectal Carcinoma

Four dried and a single fresh samples of rectal carcinoma, all removed by operation, were investigated.

1. Two of the dried samples (LK and ID) were treated in the same way as usual, and alcohol precipitate was prepared without removing the coagulum formed during evaporation. Yields of the precipitates were 300 mg from 4 g (IK) and 700 mg from 8.5 g (ID) of the original dried material.

When tested on mice IK precipitate was definitely active in 50 mg doses (2.4 and 3.6), while ID precipitate was barely so in 100 mg amounts (4.3 and 6.0).

2. Two other dried samples (OY, 8g, and YB 3.2 g) of rectal cancer were treated in the same manner as above, but the coagula formed during the concentration of extract was centrifuged off before adding absolute alcohol. Yields of alcohol precipitates were 400 mg from 8 g (OY) and 100 mg from 3.2 g (YB).

The both precipitates were found to be active, but YB precipitate was especially potent, figures being 8.0 and 4.1 for OY and 2.6 and 2.9 for YB, both in 50 mg amounts.

3. A single fresh sample of rectal cancer tissue (MS) was directly extracted with water, and the alcohol precipitate prepared in the usual manner. The coagulum was not removed. 550 mg of the alcohol precipitate was obtained from 28 g of tne original fresh material.

This precipitate was active in 100 mg doses; the figures being 3.3 and 3.5.

Sigmoid Carcinoma

There were available two cases of the carcinoma of the sigmoid region, surgical materials. One gave a very fatty dried material, which had to be well washed with ether before being subjected to extraction with water. All the procedures were the same as in the preceding. The coagulum was not removed before adding alcohol. 250 mg of the alcohol precipitate was obtained from 4 g of the original dried material.

The precipitate was highly active, the figures being 3.0 and 3.1 after injections in 50 mg amounts.

The other material (YM), 4.5 g, yielded 250 mg of alcoholic precipitate, coagula being removed in this case. This precipitate was less active than the first, giving 5.2 and 3.8 figures at 100 mg level.

Carcinoma of the Transverse Colon

A single case of the carcinoma of the transverse colon (KK) was available for study, a surgical case. 250 mg of the alcoholic precipitate was obtained from 4 g of dried material, coagula formed during the concentration of water extract having been removed. The precipitate was found to be active.

Two mice were injected with 100 mg each of the alcoholic precipitate. One died overnight, but the other survived in good physical condition, giving 4.0 cc value of the liver catalase activity.

Metastasis of Liver Cancer

Material (surgical) consisted of a large supra-clavicular lymph node metastasis of liver cancer (MH), histologically diagnosed as carcinoma simplex medulare. 9 g of dried material gave 550 mg of the alcoholic precipitate, in the preparation of which the coagula having been removed.

The alcoholic precipitate was injected into two mice in 100 mg amounts: one died overnight and the other survived. The figure for this latter was 3.8, which showed that the precipitate was active.

Bladder Carcinoma

This material also came from an autopsy case (KT). 16 g of dried material yielded 800 mg of the alcoholic precipitate, excluding the coagula.

It proved to be active when injected in 100 mg amounts, the figures being 2.9 and 4.0.

Mammary Carcinoma

Three samples of mammary carcinoma were available, all being surgical cases (MO, TK, and YA). Dried materials, 5.5 g, 8 g, and 10 g, were treated as usual, coagula being removed, yielding 300 mg, 400 mg, and 400 mg, respectively of alcoholic precipitates.

All the precipitates proved to be active as follows: 3.8 for MO precipitate, at 100 mg, 2.5 and 2.8 for TK at 50 mg, and 5.5 and 4.1 for YA at 100 mg levels.

Mammary Fibroadenoma

10 g of dried material (SK) was used: coagula was not removed. Yield of the alcoholic precipitate: 400 mg.

The precipitate was dubiously active at 100 mg level, figures being 4.4 and 4.8.

This is the only non-malignant tumor we so far tested. The tumor was removed by operation. It was a very large tumor and was clinically suspected of being possibly sarcomatous, but the diagnosis of fibroadenoma was histologically

established. The date are included here for what they are worth, without comment.

Melano-sarcoma

A large melano-sarcoma of the femur (TN), a surgical case. Dried material amounted to 47 g. The alcoholic precipitate obtained (coagula removed) was slightly darker in color than the samples from other sources. Yield: 2,800 mg.

This precipitate was found to be quite active, figures being 2.2 and 2.9 after injections in 50 mg amounts.

Reticulo-sarcoma

A large amount of material was obtained from a patient (TH), who happened to come to autopsy before the radiological treatment was properly instituted. 10 g only of the large amount of the dried material was used, yielding 700 mg of the alcoholic precipitate (coagula removed).

The precipitate was active at 100 mg level, figures being 4.2 and 4.4.

Summary of the Results

The foregoing experiments may be sufficient to establish the existence in malignant tissues of a substance which, when injected into animals, brings about a marked depression of the liver catalase activity in these animals. Positive results were obtained in all the malignant tissues examined, carcinoma was well as sarcoma, primary as well as metastatic growths, and surgical as well as autopsy materials.

The entire results may conveniently be tabulated together for ready inspection (Table 3).

It may be noted in these results that there are some variation in the activity of different samples of the alcoholic precipitates. Some samples were highly active in 50 mg amounts; with others the amount had to be increased to 100 mg in order to produce the same degree of effect; while there were a few exceptional samples which did not give very clean-cut effect even in 100 mg doses. There was a single sample (Gastric cancer ID) which definitely failed to affect the liver catalase activity. As to this particular sample we already stated that an active fraction was recovered from it by re-extracting it with water, however. See descriptions under the head of Gastric Carcinoma.

Whether or not the apparent difference in the activity of various samples is due to the actual difference in the toxohormone contents amongs different individual tumors cannot now be determined, although such may proved to be the case. It must not be lost sight of, however, that slight differences in the processes of preparing the fraction may have an appreciable effect on the quality of the final precipitate.

Difference in the activity of various samples of the precipitate cannot be correlated to the kinds of tumor tissue from which the samples were derived, it being impossible to say that any one type of malignant growth yields more potent precipitate than others. It seems reasonable to expect that a relation between the histological character and toxohormone content of cancer tissue may exist, but this aspect of the subject must be left for future investigation.

Table 3. Summary of the Effect of Alcoholic Precipitates of Tumor Tissues

Original Material	Amount injected mg	Mouse No.	Liver Catalase Activity (Oxygen cc)
Gastric carcinoma (TT)	25	1	2.3
		2	4.6
		3	4.8
	50	4	2.8
		5	3.3
,, ,, (HD)	50	6	3.0
		7	3.3
,, ,, (YS)	50	8	3.6
		9	4.2
		10	4.6
,, ,, (HT)	50	11	3.6
		12	10.8
,, ,, (5 cases pooled)	50	13	4.1
		14	4.3
	100	15	2.4
		16	2.8
,, ,, (TH)	100	17	4.6
		18	5.5
,, ,, (ID)	100	19	6.4
		20	7.8
,, ,, (KB)	100	21	2.8
		22	5.3
,, ,, (YB)	100	23	3.3
		24	7.9
,, ,, (HR)	100	25	3.7
		26	4.2
,, ,, Liver Metastasis (MA)	50	27	2.8
		28	2.7
Rectal Carcinoma (IK)	50	29	2.4
		30	3.6
,, ,, (ID)	100	31	4.3
		32	6.0
,, ,, (OY)	50	33	4.1
		34	8.0

,, ,, (YB)	50	35 36	2.6 2.9
,, ,, (MS)	50	37 38	5.0 5.8
	100	39 40	3.3 3.5
Sigmoid Carcinoma (KI)	50	41 42	3.0 3.1
,, ,, (YM)	100	43 44	3.8 5.2
Transverse Colon Carcinoma (KK)	100	45	4.0
Liver Carcinoma, Lymph Node Metastasis (MH)	100	46	3.8
Bladder Carcinoma (KT)	100	47 48	2.9 4.0
Mammary Carcinoma (MO)	100	49	3.8
,, ,, (TK)	50	50 51	2.5 2.8
,, ,, (YA)	100	52 53	5.5 4.1
Mammary Fibroadenoma (SK)	100	54 55	4.4 4.8
Melano-sarcoma (TN)	50	56 57	2.2 2.9
Reticulo-sarcoma (TH)	100	58 59	4.2 4.4

CONTROL EXPERIMENTS

Alcohol Precipitate from Normal Tissues

Having established the existence in malignant tissues of a toxic substance which can be assayed by its action *in vivo* on the liver catalase activity, the next step in our investigation should be to determine if the same substance can be separated from normal tissues.

In the following experiments we prepared alcoholic precipitates from normal human tissues according to the same method which enabled us to obtain the active fraction from tumors and tested their effect on the liver catalase activity.

Material included normal gastric and intestinal tissues adjacent to cancer removed at the same time by operation, and also from several cases of gastric ulcer. These materials, either previously dried or fresh, were extracted with distilled water, extract concentrated to about 1/10 volume, and treated with twice

the volume of absolute alcohol, exactly as in the case of cancer tissues already described. The precipitate was dried and washed with ether, the yield amounting to 3-5 percent of the original dry matter. In appearance this precipitate was generally darker in color and somewhat lighter in weight than that from cancer tissue. Each sample was tested in 100 mg amounts.

In spite of the fact that the injections produced much discomfort and debility in mice, the liver catalase activity was not markedly affected, as may be evident from Table 4.

In view of the fact that the activity of the alcohol precipitate from cancer tissue depends upon its water soluble matter, we also separated this fraction from the alcohol precipitate from normal tissues, 4 g of pooled precipitate yielding 1.1 g of water soluble fraction. Testing on 3 mice in 100 mg doses this sample was found to be entirely devoid of effect on liver catalase activity, figures being 8.7, 7.9 and 6.6.

Table 4. Effect of Alcohol Precipitate of Normal Tissues

Original Material	Mouse No.	Catalase Activity (Oxygen cc)
Stomach (HT, with gastric carcinoma)	1	12.6
	2	9.9
,, (TH, ,, ,, ,,)	3	10.8
	4	5.8
,, (KB, ,, ,, ,,)	5	10.3
	6	6.9
,, (YB, ,, ,, ,,)	7	8.0
	8	6.9
,, (UD, ,, ,, ,,)	9	6.5
	10	5.9
,, (HD, ,, ,, ,,)	11	5.3
,, (SK, ,, ,, ,,)	12	5.0
Rectum (MS, ,, rectum ,,)	13	8.3
	14	4.9
Sigmoid flexure (KI, ,, sigmoid ,,)	15	7.0
,, (IK, ,, rectum ,,)	16	5.8
Stomach (TY, with gastric ulcer)	17	11.5
	18	10.3
,, (YZ, ,, ,, ,,)	19	6.0
	20	4.6
,, (SM, ,, ,, ,,)	21	5.9
	22	5.4
,, (TH, ,, ,, ,,)	23	5.9
	24	4.7
Total average		7.2
Average for cancer cases		7.5
,, ,, ulcer cases		6.8

"Toxohormone Fraction" of Normal Tissue Necrosate

The necrotic products of normal tissues are known to be toxic, although neither the nature of the toxic effect nor the chemical properties of the toxic substance have been adequately investigated. In order to help defining more clearly the origin and significance of cancer toxohormone, studies have been undertaken on the effect of necrotic products.

It is possible that liver catalase may be somewhat reduced in association with the severe general intoxication due to toxic necrosate. The crucial point here seems to be whether or not the tissue necrosate contains the substance which can be identified with, or shown to be closely related to, cancer toxohormone. This question may be definitely answered by fractionating normal tissue necrosate according to the method for toxohormone and by testing the effect of the fractions upon the liver catalase activity.

In the following experiment liver, kidney and spleen from two normal rabbits were mashed together and sent through a wire mesh, and a large amount of the tissue material so prepared was injected subcutaneously on the back into two normal rabbits.

The rabbits were killed 3 days after the injection, when they showed marked signs of general intoxication, and the masses of necrotized tissue were recovered and dried in an open dish over hot water bath.

The dried necrosate material (27 g) was extracted with distilled water under heating in water bath for a few hours. After standing overnight, the extract was separated, concentrated to about 1/10 volume over hot water bath, precipitates and coagula centrifuged off, and to the brown colored supernatant solution was added twice the volume of absolute alcohol. The whitish precipitate formed was separated by centrifugation, dried and washed with ether. Yield: 800 mg.

Alcoholic solution, or the supernatant, was also dried and washed with ether, yielding amber colored hygroscopic material, 1,700 mg.

Both precipitate (toxohormone fraction) and supernatant were taken up in distilled water and injected intraperitoneally into normal mice in 100 mg amounts. Many of the injected mice died overnight; those surviving were killed by exsanguination 20 hours after the injection, and the catalase activity of liver was determined exactly according to the method already described. The results were as follows:

Table 5. Effect of Alcohol Precipitate and Filtrate from Normal Tissue Necrosate

Material injected	Nouse No.	Liver catalase activity. Oxygen cc
Alcohol precipitate	1	(Died overnight)
	2	(,, ,,)
	3	(,, ,,)
	4	(,, ,,)
	5	(,, ,,)
	6	(,, ,,)
	7	5.6
	8	5.8
Alcohol filtrate	9	(Died overnight)
	10	(,, ,,)
	11	(,, ,,)
	12	(,, ,,)
	13	(,, ,,)
	14	(,, ,,)
	15	4.6
	16	5.7
	17	6.0
	18	8.0
Untreated controls	19	5.3
	20	5.3
	21	5.7
	22	6.7
	23	8.0

Products of normal tissue necrosis, as may be expected, were found to contain fairly toxic substance, 100 mg being apparently the largest amount of either fraction that can be given without killing all the mice overnight. In spite of this high toxicity neither of the fractions produced a marked lowering of the liver catalase activity in the mice that survived. Since the alcoholic precipitate is the fraction in which toxohormone becomes concentrated, if the starting material were cancer tissue, the lack of the effect on liver catalase in this same fraction prepared from normal tissue necrosate would indicate the practical absence of the toxohormone principle in this material.

Necrotic and Non-necrotic Cancer Tissues as the Sources of Toxohormone

The preceding experiments seem sufficient to show that the products of normal tissue necrosis do not include the substance which can be identified with toxohormone, associating the latter more specifically with malignant tissues in exclusion of normal tissues. The question now confronting us is whether or not toxohormone is a product of the necrosis of malignant tissue.

In the following experiment we attempted to obtain some direct evidence concerning this point by comparing the toxohormone contents of cancer tissues macroscopically practically free from necrosis and of those containing abundance of necrotic material.

Four types of cancer tissue materials were available for this comparison: (1) Gastric cancer free from necrosis and (2) extensively infiltrated with necrotic material, and (3) practically necrosis-free rectal cancer and (4) markedly necrotic sample of the same type of cancer. From these materials the toxohormone fraction was separated by our standard process and under the identical conditions. The yields of the alcoholic precipitates were as follows:

550 mg from 11 g necrosis free gastric cancer, 250 mg from 5 g gastric cancer with necrosis, 100 mg from 3.2 g necrosis free rectal cancer, and 400 mg from 8 g "necrotic" rectal cancer.

The toxohormone activity of each preparation was tested by injecting it into normal mice in 50 and 100 mg amounts, according to our usual method. The following results were obtained:

Table 6. Comparative activities of toxohormone fraction obtained from necrotic and non-necrotic cancer tissues

Original material	Liver catalase activity (oxygen cc)			
	Injected with 50 mg		Injected with 100 mg	
Non-necrotic Gastric cancer	Mouse No. 1	7.8	Mouse No. 9	4.0
	2	6.1		
Necrotic Gastric cancer	3	9.7	10	4.5
	4	5.7		
Non-necrotic Rectal cancer	5	2.6		
	6	2.9		
Necrotic Rectal cancer	7	8.6		
	8	4.3		

The above results fail to show a perceptible difference as to the toxohormone activity between the preparations obtained from necrotic and non-necrotic cancer tissues. That necrotic cancer tissue yields no more active toxohormone fraction than any average sample of malignant tissues relatively free from necrosis may be apparent also from a comparison of the above figures with those we already quoted for a series of twenty-eight samples of various malignant tissues (Table 3).

It should also be pointed out that the yields of the toxohormone fraction are no greater from necrotic cancer tissues than from non-necrotic ones.

Comparison between Fresh and Decomposed Cancer Tissues

In order to test further the possibility that toxohormone may be engendered

as the result of necrosis and decomposition of cancer tissue, an experiment was carried out on the effect of decomposition *in vitro*.

A gastric cancer tissue removed at operation was divided into two portions: one was dried immediately while fresh, and the other portion after standing at 37°C for 20 hours, when tissue decomposition was well in progress. Alcoholic precipitates were prepared from both materials under the identical conditions and according to our established method. Fresh-dried material, 3.6 g, yielded 150 mg alcoholic precipitate; decomposed-dried material, 3.7 g, 200 mg precipitate.

The effects of these precipitates on the liver catalase activity were compared at 50 mg and 100 mg levels with the following results:

Table 7. Comparative toxohormone activities of toxohormone fractions from fresh and decomposed cancer tissues

Original material	Liver catalase activity (oxygen cc)	
	Injected with 50 mg	Injected with 100 mg
Fresh cancer tissue	7.6	3.0
Decomposed cancer tissue	7.0	3.5

The decomposed cancer tissue precipitate was no more active than the fresh tissue precipitate. It is quite clear that the toxohormone content of cancer tissue is not increased after decomposition at 37°C for 20 hours.

Whatever be the nature of the toxic substance or substances produced by normal tissues as they undergo necrosis (necrotoxin?), we are not here dealing with a substance which can be identified with the toxohormone principle of malignant tissue. This latter is characterized by certain chemical properties and is assayable by its effect on the liver catalase activity, and in the above series of experiments we failed to demonstrate the existence of such a substance as this among the necrotic products of normal tissues. It has also been shown that cancer tissues containing much necrotic materials are no better source of toxohormone than the average cancer tissues relatively free from necrosis.

These results may be taken to confirm the conclusion that toxohormone may be a product of living cancer cells.

CHEMICAL PROPERTIES

It is premature to make any definite suggestion as to the chemical nature of the toxohormone principle. The basic facts involved in the method of separating the potent fraction, as fully described earlier in this paper, demonstrate that the active substance is thermostable, non-heat coagulable, readily soluble in water but not in ether, and is precipitated from water solution by twice the volume

of absolute alcohol. It is conceivable that substance may be associated with non-coagulable protein or polysaccharide fraction. This last possibility, however, must be rejected, as we shall show later in this paper.

Solubility in Water

Toxohormone is readily soluble in water, but it seems important to determine if cancer tissue can be exhausted of toxohormone by a single extraction.

In the following experiment we subjected one and the same cancer tissue material to repeated extractions with water. This experiment, somewhat to our surprise, revealed that cancer tissue residue, after the extraction of the toxohormone fraction, yields a fairly toxic substance, which, however, shows no effect on the liver catalase activity, thus differing entirely from the toxohormone principle.

Cancer tissues from several sources were dried over hot water bath and the dried materials were pooled. 25 g of the pooled material was extracted with 500 cc of distilled water by heating in boiling water bath for a few hours, and allowed to remain at room temperature overnight, after which the extract was separated, concentrated down to 1/10 volume, and precipitated with twice the volume of absolute alcohol as usual. This extraction yielded 1.3 g of the alcohol precipitable fraction.

The residue after the removal of the first extract was re-extracted with water under the identical condition for a second time, and alcohol precipitate was prepared in the same way. This second extract yielded 1.7 g of the precipitate.

The residue after the second extraction was again extracted and alcohol precipitate similarly prepared, the yield of the precipitate amounting to 2.5 g.

The alcohol precipitates from first to third extracts were injected into normal mice and the effect on the liver catalase was tested according to the usual method. The following results were obtained.

Table 8. Extractability of Toxohormone with water

	Amount injected mg.	Mouse No.	Liver catalase activity. Oxygen cc
1st extract	100	1	3.7
		2	4.5
2nd extract	100	3	6.2
		4	8.8
3rd extract	100	5	(Died overnight)
		6	(,, ,,)
	50	7	(Died overnight)
		8	11.8
Normal control		9	10.5
		10	10.7
		11	8.1

The above results indicate that a single extraction may remove toxohormone from cancer tissue material almost completely, a second extraction being quite useless. By further extraction cancer tissue may be made to yield a substance which is far more toxic than is the toxoehrmone fraction and which is without effect on liver catalase activity. Apparently cancer tissue contains another toxic substance quite different from our toxohormone. The significance of this second toxic substance is not clear, but the fact that it is not readily soluble may suggest that it may be relatively unimportant in the chemical pathology of neoplastic disease.

Attempts at Further Concentration

We may be permitted to quote here some experiments in which we attempted to obtain further concentration and purification of toxohormone from the active alcohol precipitate which we have dealt with so far. In these experiment we took the alcohol precipitate of tested potency and reprecipitated it with acetic acid and alcohal, and found alcohol to be the more suitable precipitant upon the whole.

A lot of alcohol precipitate obtained by the usual method from gastric cancer tissue was used. The amount of the precipitate was 2.1 g, yield from 29 g of dried cancer tissue. This precipitate, tested at 100 mg doses on four mice, gave the liver catalase activity of 7.8, 6.4, 6.0 and 4.3 cc values—not a very active preparation.

1.7 g of the precipitate was thoroughly extracted with distilled water under heating, and 1400 mg of water soluble and less than 150 mg of insoluble matters were obtained. This water soluble fraction, tested at 100 mg doses was much more potent than the original alcohol precipitate, figures obtained on four mice being 5.6, 5.6, 4.2 and 3.8 cc. The insoluble fraction at 100 mg gave 6.4 cc.

950 mg of this water soluble fraction was dissolved in 100 cc of distilled water. The pale yellow solution obtained was brought to pH 3.5 by adding acetic acid and left in an ice box overnight. The white precipitate formed was recovered by centrifugation, washed with 100 cc of distilled water and dried. Yield: 250 mg.

The supernatant after the removal of the precipitate, was neutralized with NaOH, concentrated to 30 cc volume, and 60 cc of absolute alcohol added, and the precipitate formed was separated, washed with 70 % alcohol and dried. Yield: 250 mg.

Both acetic acid and alcohol precipitates obtained as above were tested as to their toxohormone potencies with the following results:

Table 9. Effect of acetic acid and alcohol precipitates

	Amount injected mg	Mouse No.	Liver catalase activity. Oxygen cc
Acetic acid precipitate	50	1	3.3
		2	4.0

	25	3 4	8.0 5,8
Alcohol precipitate	50	5 6	3.2 5.8
	25	7 8	6.0 6.7
Normal controls		9 10 11	8.8 8.8 9.3

It may be said that by re-precipitation with acetic acid approximately two fold concentration of toxohormone was obtained, as may be clear from the following tabulation:

Original alcohol precipitate (Activity dubious at 100 mg)
↓
Water extract (Mildly active at 100 mg)
↓
Acetic acid precipitate (Distinctly active at 50 mg)
Acetic acid filtrate
↓
Alcohol precipitate (Distinctly active at 50 mg)

It is also clear, however, that acetic acid precipitated only about one-half of toxohormone contained in the water extract, since an equally active fraction was obtained by precipitating acetic acid filtrate with alcohol.

In view of these facts next experiment was planned to test the efficiency of the combined use of acetic acid and alcohol for the concentration of toxohormone.

20 g of another pooled sample of dried gastric cancer tissue was extracted with distilled water and alcohol precipitate prepared in the same manner as before. Yield of alcohol precipitate: 1.3 g.

1 g of the precipitate was extracted with 60 cc of distilled water under heating, and to this extract was added first sufficient acetic acid to bring the reaction to pH 3.5, and then twice the volume of absolute alcohol. The precipitate produced was dried, washed with absolute alcohol and ether. Yield: 250 mg.

The two precipitates were tested as usual for their toxohormone activities, and the results were as follows:

Table 10. Effect of Alcohol and Alcohol-Acetic acid Precipitates

	Amount injected mg	Mouse No.	Liver catalase activity. Oxygen cc
1st precipitate(Alcohol)	100	1 2	5.6 3.8
	50	3 4	5.5 6.8
2nd precipitate (Alcohol and acetic acid)	50	5 6	3.7 4.3

	25	7 8	4.2 4.5
Normal controls		9 10	8.7 11.6

By the combine use of alcohol and acetic acid it was possible to obtain a toxohormone fraction active at 25 mg level from the original alcohol precipitate which was fairly active at 100 mg and definitely inactive at 50 mg.

Nucleo-Protein Fraction

The behavior of toxohormone to acetic acid does not seem to indicate that we are dealing with nucleo-protein. Nevertheless, a brief description of our experiment on this fraction may be permissible here.

In the following experiment we separated from one and the same cancer tissue extract three different fractions, one precipitated with acetic acid at pH 3.5, another with calcium chloride, and third with alcohol, and compared their toxohormone potencies.

20 g of pooled samples of gastric cancer desiccates was extracted with 800 cc of distilled water, heated for an hour or so, according to the usual method.

300 cc of the extract, completely free of precipitate and coagula, was brought to pH 3.5 by acetic acid and kept at 37°C for a few minutes, the precipitate formed was dissolved with NaOH and reprecipitated with acetic acid at pH 3.5. The final precipitate was washed with distilled water, dried, and washed with absolute alcohol and ether. Yield of this nucleo-protein fraction: 150 mg.

100 cc of the same extract was precipitated by adding 15 cc of 40 percent solution of calcium chloride and warming to 37°C, and the precipitate was washed with distilled water, dried, and washed with absolute alcohol and ether. Yield of this nucleohistone fraction: 50 mg.

The remaining extract, 400 cc in amount, was precipitated, after evaporation to 40 cc, by adding 80 cc of absolute alcohol, according to our standard method for obtaining the toxohormone concentrate. Yield of alcohol precipitate: 400 mg.

The toxohormone activity of the three kinds of precipitates obtained as above was tested as usual, and the results were as follows:

Table 11. Effett of Nucleo Protein Fractions

	Amount injected mg	Mouse No.	Liver catalase activity. Oxygen cc
Nucleo-protein fraction	50	1 2	5.9 6.2
Nucleo-histone fraction	50	3	5.7

Alcohol precipitate	50	4 5	4.2 3.4
Normal control	0	6	5.8

Polysaccharide Fraction

With the possibility in mind that toxohormone may be recoverable as a polysaccharide fraction we next prepared such a fraction according to Boivan's method frequently used for the separation of bacterial polysaccharide.

8 g of pooled cancer tissue desiccate (gastric and rectal cancers) was extracted with 150 cc of 3 percent solution of trichloroacetic acid, and after overnight in an ice box the extract was separated by centrifugation, and the opaque, whitish supernatant obtained was dialysed against cold running water for 48 hours.

After centrifuging off the floccula formed during dialysis the clear solution (pH 6.0) was evaporated to 20 cc, mixed with 60 cc of acetone, and the precipitate formed was recovered by centrifugation. Yield: 400 mg. The yield of the floccula formed during dialysis was 60 mg; the solids of acetone soluble supernatant 150 mg.

The toxohormone activity of the acetone precipitate was tested according to our usual method, along with those of other fractions incidentally obtained. The results were as follows:

Table 12. Effect of Polysaccharide and Other Fractions

	Amount injected mg	Mouse No.	Liver catalase activity. Oxygen cc.
Acetone precipitate	100	1 2	(Died overnight) (,, ,,)
	50	3 4	11.3 10.6
Acetone filtrate	150	5	(Died in 1 hour)
Floccula formed during dialysis	60	6	13.3
Normal control	0	7	9.2

It seems that the polysaccharide fraction obtained by this method does not carry the toxohormone principle. The fraction, however, is fairly toxic to mice, and this again indicates that toxohormone activity is not associated with the toxicity which is determinable by the lethal action.

It is possible that toxohormone is inactivated by trichloroacetic acid, or is completely precipitated by it. It is also possible that toxohormone is dialysable. Any one of these possibilities can account for the inactivity of the acetone precipitate.

MODE OF ACTION

It must be clearly understood at the outset that the reduced activity of the liver catalase we are dealing with in this investigation is not associated with any morphologically recognizable injury in the liver. Any marked injury to the liver cells would naturally lead to the corresponding reduction of all biochemical activities of the liver, but decidedly such is not the case with the problem which we have before us.

Nor is it possible to confuse the toxohormone effect with the generni intoxication due to histogenic toxic substances, since there is no parallelism between the toxohormone activity and toxicity of any given tissue fraction. We found that all the highly toxic fractions separated by us to be devoid of the liver catalase depressing action, while the active toxohormone fractions were least toxic in the term of their lethal action on mice.

Effect in vitro on Liver Catalase

There are numerous chemical substances that act as inhibitors to catalase activity *in vitro*. In considering the mode of action of toxohormone on liver catalase the possibility of the substance acting as an inhibitor in this sense should be the first question to be taken up. Concerning this point Greenstein's experiments may be quoted:

Extracts of normal rat liver were incubated with extracts of rat sarcoma, and with serum and extracts of other tissues of tumor-bearing rats. Similarly, rat liver slices were incubated with tumor slices, and extracts of normal mouse liver with extracts of sarcoma. None of these mixtures affected the catalase activity of the liver. Thus, tumor tissue apparently has no direct action *in vitro* on liver catalase.

There is, however, a possibility that tumor extracts or slices used may not have contained the inhibitor in a sufficient concentration to reveal its action.

Now that we have demonstrated that the depression of the liver catalase *in vivo* is due to a peculiar toxic substance (toxohormone) produced by tumor tissue, and obtained the substance in the form of a potent fraction, it may be appropriate that *in vitro* experiments should be carried out using this potent preparation.

In the following experiment we tested the effect of a drastically large amount of our toxohormone preparation. 200 mg of a sample tested and proved to be active *in vivo* was homogenized in 3 cc of phosphate buffer solution of pH 7. Liver catalase solution was prepared from the liver of a normal mouse by grinding up 0.1 g of the liver tissue, extracting it with 10 cc of phosphate buffer of pH 7, and centrifuging off the solids at 3,000 r.p.m. for 5 minutes.

To 0.3 cc of the liver catalase solution was added 1.5 cc of the toxohormone homogenate (corresponding to 100 mg toxohormone material) or the same amount of the plain phosphate buffer for control. The mixture was allowed to stand at room temperature for 60 minutes, after which the catalase activity was determined according to our usual method. The results of duplicate experiments were as follows:

Table 13. Effect *in vitro* of toxohormone on liver catalase activity

Catalase solution cc	Plain buffer cc	Toxohormone homogenate cc	Catalase activity Oxygen cc
0.3	1.5	0	10.7
0.3	1.5	0	10.5
0.3	0	1.5	10.7
0.3	0	1.5	9.5

The fact that no material effect was observed even in so high a concentration as this (100 mg per 1.8 cc) must be taken to mean that toxohormone does not affect liver catalase *in vitro*. This renders it impossible to account for the effect *in vivo* on the basis of the direct action of toxohormone on catalase. One possible explanation may be derived from the suggestion of Greenstein, namely, that toxohormone in someway interferes with the normal synthesis of catalase.

From a broader point of view we already suggested that the effect *in vivo* on liver catalase may be but one expression of the deleterious effect on the vital function of liver cells which the action of this toxohormone may bring about. The entire picture of the deleterious effect is impossible to visualize at this time, but it must be freely admitted that there exists a considerable evidence as to the imparement of the liver function in cancer.

It may be of interest to recall here Greenstein's findings[1] that the kidney catalase activity is lowered relatively little, and blood catalase activity not at all in tumor bearing animals. The relative insusceptibility of kidney catalase may of course be due to a partial excretion of toxohormone by the kidney, and the failure of the blood catalase to be affected may be explained on the basis of the very low concentration of toxohormone in the blood. Fact remains, however, that liver catalase is far more markedly affected than catalase in other tissues, pointing to the closer implication of liver cells than other tissue cells in toxohormone effect.

CONCLUSIONS

Systemic changes found in association with cancer are generally not characteristic, and there are numerous observations indicating that "Cancer cachexia is in no way different from cachexia of other conditions."[2] So far as we know

at the present time, decrease of liver catalase is the only outstanding and clearly definable effect produced in cancer. In the present investigation a substance which accounts for this outstanding systemic change has been separated from cancer tissue.

The substance in question is thermostable, non-heat coagulable, water soluble and alcohol precipitable. It seems to be associated with non-coagulable protein. Injections of the active fraction into animals promptly bring about a marked decrease in the amount of liver catalase beyond the limits of the normal variability.

It has been possible to obtain active preparation from all the types of malignant tissues, both carcinoma and sarcoma, so far tested, but not from normal tissues or from the products of tissue necrosis and decomposition. It is clearly distinguishable from all other histogenic toxic substance, which have no special effect on liver catalase.

The substance shows no inhibiting action on catalase *in vitro*, but seems to interfere with the normal production of catalase in the liver. The decreased catalase activity may be an expression of the reduced vital funtion of the liver cell, and the cancer cell product ("toxohormone"), which thus deleteriously acts upon the liver cell, may well be one of the important underlying factors in cancer cachexia.

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References

1. Nakahara, W., and Fukuoka, F.: A toxic cancer tissue constituent as evidenced by its effect on liver catalase activity. Japan. Med. Jour., Vol. 1, 271 (1948).
2. Wells, H. G.: Chemical Pathology. (1925)
3. Franke, W.: Katalase. Handb. d. Enzymol. von Nord u. Weidenhagen. Bd. 2, 765 (1940).
4. Greenstein, J. P., Jenrette, W. V., and What, J.: The liver catalase activity of tumor bearing rats and the effect of extirpation of the tumor. Jour. Nat. Cancer Inst., Vo. 2, 283 (1941).
5. Greenstein, J. P., and Andervont, H. B.: The liver catalase activity of tumor bearing mice and the effect of spontaneous regression and removal of certain tumors. Jour. Nat. Cancer Inst., Vol. 2, 1345 (1942).
6. Greenstein, J. P.: Titration of the liver catalase activity of normal and of tumor bearing rats and mice. Jour. Nat. Cancer Inst., Vol. 2, 525 (1942).
7. Greenstein, J. P.: Further studies of liver catalase activity of tumor bearing animals. Jour. Nat. Cancer Inst., Vol. 3, 397 (1943).
8. Greenstein, J. P.: Note on the liver catalase activity of pregnant mice and of mice bearing growing embryonic implants. Jour. Nat. Cancer Inst., Vol. 4, 283 (1943).
9. Greenstein, J. P., and Andervont, H. B.: Kindney and blood catalase activity of tumor bearing animals. Jour. Nat. Cancer Inst., Vol. 2, 589 (1942).

ADDENDUM

While this paper was in press we obtained several data confirming and extending our conclusion as to the chemical nature of toxohormone.

1. Starting with a pooled sample of the alcohol precipitates, tested and proved to be active in 50-100 mg doses, we precipitated water solution of it with trichloroacetic acid and obtained the protein fraction very active in 25 mg doses.

2. A similar water solution of the alcohol precipitate was precipitated with picric acid with the addition of acetic acid (no precipitate being produced without acetic acid, thus excluding the presence of protamine), the precipitate dissolved in water and re-precipitated with alcohol, removing picric acid at the same time. The final precipitate was highly active in 25 mg doses.

3. The water solution was fractionated with ammonium sulphate. The precipitates at half saturation as well as at full saturation were active in 25 mg doses, but non-precipitable (peptone) fraction was found to kill a mouse overnight in a 50 mg dose.

4. All these active protein fractions showed a very feeble Molisch reaction: as may be expected the xanthoprotein reaction gave no yellow precipitate but only yellow solution.

Taken together, these findings would seem to indicate that toxohormone may be associated with proteose class of hydrolysed protein. The details of these experiments will appear later in this journal. —W. N. and F. F.

要　旨

癌組織の生産する特殊毒性物質（トキソホルモン）

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癌組織が何か毒性物質を生産するであろうということは，かなり古くから考えられていたのであるが，今日まで何等特殊なものが見出されていない。これは癌における全身的障害が具體的につかまれていなかったのでかような物質を探す手懸りがなかったためであると思われる。

近年になって，肝臓カタラーゼの著明な減少が，癌の全身障害の一つとしてはっきり認識されるようになった。肝カタラーゼの減少は以前から癌患者及び擔癌動物において觀察されていたのであるが，最近 Greenstein の研究によってその意義が非常に明らかになったのである。

Greenstein は擔癌動物の肝臓カタラーゼが他の酵素に比し著しく減少している點を確かめ，さらに癌を外科的に切除すると肝カタラーゼが正常に復し，再移植するとまた肝カタラーゼが減少することを明らかにした。姙娠動物では肝カタラーゼは正常であり，巨大なエムブリオームを作っても何等の影響がない。すなわち，肝カタラーゼの減少は癌に特異である。また試驗管内實驗から肝カタラーゼ作用の減少は，作用が抑制されるのではなく，カタラーゼ量が減少するのであることを證明した。

そこで癌組織が一種の物質を生產（分泌）し，それが血液を介して肝臓に至り，肝カタラーゼを減少せしめることが當然考えられるのであるが Greenstein は腫瘍エキストラクト中にかような物質があることを證明し得ず，結局動物體内に腫瘍が增殖していることが必要條件であると結論したのである。

我々は Greenstein がこの物質を證明し得なかったのは，それが癌組織中に極く少量しか含まれていないため，普通にエキストラクトを作ったのではその作用が認められないのであろうと推定した。もしさうなら，癌組織からその物質を濃縮物としてとり出せばその作用を證明し得る筈である。ここに報告した我々の實驗はそれを成し遂げたものである。

この物質は病的組織が生產する，特殊な毒作用を持った一種の内分泌物と考えることが出來る。その意味で我々は假にこれを「トキソホルモン」と命名した。

その濃縮分離方法としては，種々の豫備實驗を試みた結果，次の方法が最も確實であることを決定した。すなわち癌組織を水で浸出し，浸液を適當量に蒸發濃縮し，これに 2 倍量の純アルコールを加えて生ずる沈澱を採り，乾燥してエーテルで洗うのである。收量は原料の 5～8% が普通である。

物質の作用はそれを正常マウスに注射し，肝臓カタラーゼ作用の減少によって判定する。

正常マウスの肝カタラーゼは個體による變化が多く，我々の實驗した73例では酸素量で最高16.4，最低4.6，平均7.9となり，大多數例は6～10，5以下は極めて少數で全例の4%位であつた。

ところが癌のアルコール沈澱物50～100 mgを注射すると，20時間位で肝カタラーゼが顯著に減少する，多くの場合正常の最低値を突破し，甚しいものは2近くまでも減少する。

我々の檢した癌組織は全部人體材料で，胃癌が大部分であつたが，直腸その他の腸癌，乳癌，胃癌の肝臟轉移，肝癌の淋巴腺轉移，肉腫としては黑色肉腫，細網肉腫があつた。トキソホルモンはこれ等の總てから證明することが出來た。

對照實驗としては，癌或いは胃潰瘍患者から切除した正常胃或いは腸粘膜，及び動物の正常組織を動物體に注入して壞死に陷らしめたものから同樣の方法でアルコール沈澱物を採つたが，これ等は何れも肝カタラーゼには何等の影響をも與えるものではなかつた。

またトキソホルモンが死んだ癌組織の分解產物ではないかという點を決定するため，癌組織の特に壞死部の多いもの，或いは體外で腐敗せしめたものについて檢討したが，壞死或いは腐敗によつてトキソホルモンが增加することは全く認められなかつた。

トキソホルモンの化學的本体は未だ明らかでないが，それが耐熱性であること，加熱によつて凝固しないこと，エーテルに不溶，水によく溶け，水溶液からアルコールで沈澱することは明らかである。このような性狀から差し當り多糖類と非凝固性蛋白との關係が考えられるが，我々はむしろ後者と關聯しているものと考えている。多糖類として分離することには成功しなかつた。

この研究は單に癌組織から毒性を有する一物質を抽出したというのではない。一定量を注射して動物を殺す物質は癌組織は勿論，正常或いは壞死組織等からも容易に分離することが出來る。このような毒性物質は肝カタラーゼを減少せしめる作用を有しない。トキソホルモンとは全く別個のものである。

トキソホルモンは惡性腫瘍組織から分泌され，體液に運ばれて動物體の一定の細胞機能に障害を與えるもので，しかもその障害（肝カタラーゼの減少）が癌に於ける殆んど唯一の顯著な全身障害と認められている點に於て特別な意義を有するものである。

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